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Although it is not believed that any additional fee is required to consider this submission, the Commissioner is hereby authorized to charge our deposit account no. 04-1105 should any fee be deemed necessary.

Respectfully submitted,

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Kathryn A. Piffat, Ph.D.

Kathryn A. Piffat, Ph.D. (Reg. No. 34,901)
Dike, Bronstein, Roberts & Cushman
Intellectual Property Practice Group of
EDWARDS & ANGELL, LLP
P.O. Box 9169
Boston, MA 02209
(617) 439-4444

(40) A method for screening a compound that alters the binding property between a ligand and the protein or its salt according to (28), which comprises using the protein or its salt according to (28) or the
5 partial peptide or its amide or ester, or a salt thereof, according to (30).

(41) A kit for screening a compound that alters the binding property between a ligand and the protein or its salt according to (28), comprising the protein
10 or its salt according to (28) or the partial peptide or its amide or ester, or a salt thereof, according to (30).

(42) A compound that alters the binding property between a ligand and the protein or its salt according to (28), which is obtainable by using the screening
15 method according to (40) or the screening kit according to (41).

(43) A pharmaceutical composition comprising a compound that alters the binding property between a ligand and the protein or its salt according to (28),
20 which is obtainable by using the screening method according to (40) or the screening kit according to (41).

(44) A method for quantifying the protein or its salt according to (28), which comprises using the
25 antibody of (36).

The present invention further relates to the following:

(45) A polypeptide, its amide or ester, or a salt
30 thereof, according to (1), wherein substantially the same amino acid sequence as the amino acid sequence shown by SEQ ID NO:1 is an amino acid sequence possessing homology of at least about 70%, preferably at least about 80%, more preferably at least about 90%

Examples of the polypeptide which has substantially the same amino acid sequence as that shown by SEQ ID NO:1 include a polypeptide containing the 22-180 amino acid sequence of the amino acid sequence represented by SEQ ID NO:1, etc.

Preferred examples of the polypeptide which has substantially the same amino acid sequence as that represented by SEQ ID NO:1 include a polypeptide having substantially the same amino acid sequence as that represented by SEQ ID NO:1 (e.g., amino acid sequence shown by SEQ ID NO:8, SEQ ID NO:14, SEQ ID NO:18, SEQ ID NO:33 or SEQ ID NO:50) and having the activity substantially equivalent to that of the amino acid sequence represented by SEQ ID NO:1.

Examples of the substantially equivalent activity include a cell-stimulating activity caused by adding such a polypeptide (specifically, a protein containing the same or substantially the same amino acid sequence as that shown by SEQ ID NO:37, or its salts) to the polypeptide receptor-expressing cells (hereinafter simply referred to as a cell stimulating activity) such as arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP formation, intracellular cGMP formation, inositol phosphate production, change in cell membrane potential, phosphorylation of intracellular proteins, activation of c-fos and change in extracellular pH, etc.), a somatostatin secretion regulating activity and the like.

The term "substantially equivalent" is used to mean that the nature of these activities is equivalent (for example, biochemically or pharmacologically). Therefore, it is preferred that the these activities such as a cell-stimulating activity, a somatostatin secretion regulating activity, etc. are equivalent in strength (e.g., about 0.1 to about 100 times,

acids are added, an amino acid sequence wherein 1 to 5 (preferably 1 to 3) amino acids are inserted, or an amino acid sequence wherein 1 to 5 (preferably 1 to 3) amino acids are substituted by other amino acids. The partial peptide may contain a combination of the above amino acid sequences.

In the partial peptide of the present invention, the C-terminus is normally a carboxyl group ($-\text{COOH}$) or carboxylate ($-\text{COO}^-$) but the C-terminus may be in the form of an amide ($-\text{CONH}_2$) or an ester ($-\text{COOR}$) (wherein R has the same significance as defined above), as has been described with the polypeptide of the present invention. In particular, preferred are the partial peptides having an amide ($-\text{CONH}_2$) at the C-terminus.

As in the polypeptide of the present invention described above, the partial peptide of the present invention further includes conjugated peptides such as those in which the amino group of the N-terminal amino acid residue (e.g., methionine residue) is protected by a protecting group, those in which the N-terminal residue is cleaved in vivo and the produced glutamine residue is pyroglutaminated, those in which substituents on the side chains of amino acids in the molecule are protected by appropriate protecting groups and conjugated proteins such as so-called glycoproteins having sugar chains.

The partial peptide of the present invention can be employed as an antigen for producing an antibody and therefore, does not necessarily require the cell stimulating activity, the somatostatin secretion regulating activity, etc.

The polypeptide, amides or esters of the present invention or the partial peptide, amides or esters of the present invention may be used in the form of salts with physiologically acceptable acids (e.g., inorganic

acids or organic acids) or bases (e.g., alkali metal salts), preferably in the form of physiologically acceptable acid addition salts. Examples of such salts are salts with inorganic acids (e.g., hydrochloric acid, phosphoric acid, hydrobromic acid, sulfuric acid), salts with organic acids (e.g., acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid, benzenesulfonic acid) and the like.

The polypeptide of the present invention or salts thereof may be manufactured by a publicly known method used to purify a polypeptide from human or other warm-blooded animal cells or tissues described above. Alternatively, the polypeptide of the present invention or salts thereof may also be manufactured by culturing a transformant containing DNA encoding the polypeptide of the present invention, as will be later described. Furthermore, the polypeptide of the present invention or salts thereof may also be manufactured by the methods for synthesizing proteins, which will also be described hereinafter, or by modified methods.

Where the polypeptide or salts thereof are manufactured from human or mammalian tissues or cells, human or mammalian tissues or cells are homogenized, then extracted with an acid or the like, and the extract is isolated and purified by a combination of chromatography techniques such as reverse phase chromatography, ion exchange chromatography, and the like.

To synthesize the polypeptide of the present invention, its partial peptide or its salts or amides, commercially available resins that are used for polypeptide synthesis may be used. Examples of such resins include chloromethyl resin, hydroxymethyl resin,

benzhydrylamine resin, aminomethyl resin, 4-benzyloxybenzyl alcohol resin, 4-methylbenzhydrylamine resin, PAM resin, 4-hydroxymethylmethylphenyl acetamidomethyl resin, polyacrylamide resin, 4-(2',4'-dimethoxyphenyl-hydroxymethyl)phenoxy resin, 4-(2',4'-dimethoxyphenyl-Fmoc-aminoethyl) phenoxy resin, etc. Using these resins, amino acids in which α -amino groups and functional groups on the side chains are appropriately protected are condensed on the resin in the order of the sequence of the objective polypeptide according to various condensation methods publicly known in the art. At the end of the reaction, the polypeptide is excised from the resin and at the same time, the protecting groups are removed. Then, intramolecular disulfide bond-forming reaction is performed in a highly diluted solution to obtain the objective polypeptide, partial peptide or amides thereof.

For condensation of the protected amino acids described above, a variety of activation reagents for polypeptide synthesis may be used, but carbodiimides are particularly preferably employed. Examples of such carbodiimides include DCC, N,N'-diisopropylcarbodiimide, N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide, etc. For activation by these reagents, the protected amino acids in combination with a racemization inhibitor (e.g., HOBt, HOObt) are added directly to the resin, or the protected amino acids are previously activated in the form of symmetric acid anhydrides, HOBt esters or HOObt esters, followed by adding the thus activated protected amino acids to the resin.

Solvents suitable for use to activate the protected amino acids or condense with the resin may be chosen from solvents that are known to be usable for polypeptide condensation reactions. Examples of such

flow in the presence of a catalyst such as Pd-black or Pd-carbon; an acid treatment with anhydrous hydrogen fluoride, methanesulfonic acid, trifluoromethanesulfonic acid or trifluoroacetic acid, or a mixture solution of these acids; a treatment with a base such as diisopropylethylamine, triethylamine, piperidine or piperazine; and reduction with sodium in liquid ammonia. The elimination of the protecting group by the acid treatment described above is carried out generally at a temperature of approximately -20°C to 40°C. In the acid treatment, it is efficient to add a cation scavenger such as anisole, phenol, thioanisole, m-cresol, p-cresol, dimethylsulfide, 1,4-butanedithiol or 1,2-ethanedithiol. Furthermore, 2,4-dinitrophenyl group known as the protecting group for the imidazole of histidine is removed by a treatment with thiophenol. Formyl group used as the protecting group of the indole of tryptophan is eliminated by the aforesaid acid treatment in the presence of 1,2-ethanedithiol or 1,4-butanedithiol, as well as by a treatment with an alkali such as a dilute sodium hydroxide solution and dilute ammonia.

Protection of functional groups that should not be involved in the reaction of the starting materials, protecting groups, elimination of the protecting groups and activation of functional groups involved in the reaction may be appropriately selected from publicly known groups and publicly known means.

In another method for obtaining the amides of the polypeptide or partial peptide of the present invention, for example, the α -carboxyl group of the carboxy terminal amino acid is first protected by amidation; the peptide (polypeptide) chain is then extended from the amino group side to a desired length. Thereafter, a polypeptide in which only the protecting group of the

The protein which has substantially the same amino acid sequence as that shown by SEQ ID NO:37 is preferably a protein having substantially the same amino acid sequence shown by SEQ ID NO:37 and having
 5 the activities substantially equivalent to the amino acid sequence shown by SEQ ID NO:37. A specific example of such protein is a protein containing the amino acid sequence represented by SEQ ID NO:54.

The substantially equivalent activities are, for
 10 example, a ligand binding activity, a signal transduction activity, a somatostatin secretion regulating activity, etc. The term "substantially equivalent" is used to mean that the nature of these activities is equivalent. Therefore, it is preferred
 15 that these activities such as ligand binding activity, a signal transduction activity, a somatostatin secretion regulating activity, etc. are equivalent in strength (e.g., about 0.1 to about 100 times, preferably about 0.5 to about 20 times, more preferably
 20 about 0.5 to about 2 times), and it is allowable that even differences among grades such as the strength of these activities and molecular weight of the polypeptide are present.

The activities such as a ligand binding activity,
 25 a signal transduction activity, a somatostatin secretion regulating activity or the like can be assayed according to a publicly known method, for example, by means of ligand determination or screening, which will be later described.

30 The receptor protein of the present invention which can be employed include proteins comprising (i) an amino acid sequence represented by SEQ ID NO:37 or SEQ ID NO:54, of which at least 1 or 2 (preferably 1 to 30, more preferably 1 to 10 and most preferably several
 35 (1 or 2)) amino acids are deleted; (ii) an amino acid

COO⁻) but may be in the form of an amide (-CONH₂) or an ester (-COOR), as in the polypeptide of the present invention described above.

Furthermore, examples of the partial peptide of the receptor protein in the present invention include variants of the above peptides, wherein the amino group at the N-terminal methionine residue is protected with a protecting group, those wherein the N-terminal region is cleaved in vivo and the Gln formed is pyroglutaminated, those wherein a substituent on the side chain of an amino acid in the molecule is protected with a suitable protecting group, or conjugated proteins such as glycoproteins having sugar chains, as in the receptor protein of the present invention described above.

As the salts of the receptor protein or its partial peptide in the present invention, physiologically acceptable acid addition salts are particularly preferred. Examples of such salts are salts with inorganic acids (e.g., hydrochloric acid, phosphoric acid, hydrobromic acid, sulfuric acid), salts with organic acids (e.g., acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid, benzenesulfonic acid) and the like.

The receptor protein or salts of the present invention may be manufactured by a publicly known method used to purify a receptor protein from human or other mammalian cells or tissues described above, or by preparing a transformant containing the DNA encoding the receptor protein of the present invention (a host similar to the host of the transformant containing the DNA encoding the polypeptide of the present invention described above may be used) in a manner similar to the

aforesaid method for preparing the transformant containing the DNA encoding the polypeptide of the present invention, culturing the resulting transformant in a manner similar to the aforesaid method for preparing the transformant containing the DNA encoding the polypeptide of the present invention. Furthermore, the receptor protein or salts of the present invention may also be manufactured by the aforesaid methods for synthesizing polypeptides or by modified methods.

Where the receptor protein or salts thereof are manufactured from human or mammalian tissues or cells, human or mammalian tissues or cells are homogenized, then extracted with an acid or the like, and the extract is isolated and purified by a combination of chromatography techniques such as reverse phase chromatography, ion exchange chromatography, and the like.

The partial peptide of the receptor protein or salts thereof in the present invention can be manufactured by a publicly known method used to synthesize a peptide or, by cleaving the receptor protein of the present invention with an appropriate peptidase.

The receptor protein or salts of the present invention, its partial peptide, amides, esters or salts can be synthesized by the aforesaid method for synthesizing the polypeptide, amides, esters or salts of the present invention.

For the polynucleotide encoding the receptor protein of the present invention, any polynucleotide can be used as long as it contains the base sequence (DNA or RNA, preferably DNA) encoding the receptor protein of the present invention. Such a polynucleotide may be DNA and RNA including mRNA encoding the receptor protein of the present invention. The polynucleotide

may be double-stranded or single-stranded. Where the polynucleotide is double-stranded, it may be double-stranded DNA, double-stranded RNA or DNA:RNA hybrid. Where the polynucleotide is single-stranded, it may be
 5 a sense strand (i.e., a coding strand) or an antisense strand (i.e., a non-coding strand).

Using the polynucleotide encoding the receptor protein of the present invention, mRNA of the receptor protein of the present invention can be quantified by,
 10 for example, the publicly known method published in separate volume of *Jikken Igaku* 15 (7) "New PCR and its application" (1997) or the modified method.

The DNA encoding the receptor protein of the present invention may be any of genomic DNA, genomic
 15 DNA library, cDNA derived from the cells and tissues described above, cDNA library derived from the cells and tissues described above and synthetic DNA. The vector to be used for the library may be any of bacteriophage, plasmid, cosmid and phagemid. The DNA
 20 may also be directly amplified by reverse transcriptase polymerase chain reaction (hereinafter abbreviated as RT-PCR) using the total RNA or mRNA fraction prepared from the cells and tissues described above.

Specifically, the DNA encoding the receptor
 25 protein of the present invention may be any DNA having the base sequence shown by SEQ ID NO:38, SEQ ID NO:55 or SEQ ID NO:56, or the base sequence hybridizable to the base sequence represented by SEQ ID NO:38, SEQ ID NO:55 or SEQ ID NO:56 under high stringent conditions
 30 and encoding a polypeptide which has the activities substantially equivalent to those of the receptor protein of the present invention (e.g., a ligand binding activity, a signal transduction activity or a somatostatin secretion regulating activity, etc.).

Specific examples of the DNA that is hybridizable to the base sequence represented by SEQ ID NO:38, SEQ ID NO:55 or SEQ ID NO:56 include DNA having at least about 70% homology, preferably at least about 80% homology, more preferably at least about 90% homology and most preferably at least about 95% homology, to the base sequence represented by SEQ ID NO:38, SEQ ID NO:55 or SEQ ID NO:56.

The hybridization can be carried out by publicly known methods or by a modification thereof, for example, according to the method described in Molecular Cloning, 2nd Ed., J. Sambrook et al., Cold Spring Harbor Lab. Press, (1989). A commercially available library may also be used according to the instructions of the attached manufacturer's protocol. The hybridization can be carried out preferably under high stringent conditions.

The high stringent conditions used herein refer to the conditions, for example, in a sodium concentration of about 19 mM to about 40 mM, preferably about 19 mM to about 20 mM at a temperature of about 50°C to about 70°C, preferably about 60°C to about 65°C. In particular, hybridization conditions in a sodium concentration of about 19 mM at a temperature of about 65°C are most preferred.

The polypeptide encoded by the DNA, which is hybridizable to the base sequence shown by SEQ ID NO:38, SEQ ID NO:55 or SEQ ID NO:56 can be manufactured by methods similar to those for manufacturing the polypeptide of the present invention, described above. Examples of the amides, esters or salts of the polypeptide are the same as those for the amides, esters or salts of the polypeptide of the present invention described above.

More specifically, for the DNA encoding the receptor protein having the amino acid sequence represented by SEQ ID NO:37, DNA having the base sequence represented by SEQ ID NO:38 may be employed;
 5 and DNA having the base sequence represented by SEQ ID NO:55 or SEQ ID NO:56 may be used for the DNA encoding the receptor protein having the amino acid sequence represented by SEQ ID NO:54.

10 The polypeptide containing a part of the base sequence of DNA encoding the receptor protein of the present invention or a part of the base sequence complementary to the DNA is used to mean that not only the DNA encoding the partial peptide of the present invention described below but also RNA are embraced.

15 According to the present invention, antisense polynucleotides (nucleic acids) that can inhibit replication or expression of the G protein-coupled receptor protein gene can be designed and synthesized based on the cloned or determined base sequence
 20 information of the DNA encoding the G protein-coupled receptor protein. Such a polynucleotide (nucleic acid) is capable of hybridizing with RNA of G protein coupled receptor protein gene to inhibit the synthesis or function of said RNA or capable of modulating the
 25 expression of a G protein-coupled receptor protein gene via interaction with G protein coupled receptor protein-associated RNA. Polynucleotides complementary to selected sequences of RNA associated with G protein-coupled receptor protein and polynucleotides
 30 specifically hybridizable with the selected sequences of RNA associated with G protein-coupled receptor protein are useful in modulating or controlling the expression of a G protein coupled receptor protein gene in vivo and in vitro, and in treating or diagnosing
 35 disease later described. The term "corresponding" is

used to mean homologous to or complementary to a particular sequence of the base sequence or nucleic acid including the gene. The term "corresponding" between nucleotides, base sequences or nucleic acids and peptides (proteins) usually refers to amino acids of a peptide (protein) under the order derived from the sequence of nucleotides (nucleic acids) or their complements. 5' end hairpin loop, 5' end 6-base-pair repeats, 5' end untranslated region, polypeptide translation initiation codon, protein coding region, ORF translation initiation codon, 3' untranslated region, 3' end palindrome region, and 3' end hairpin loop in the G protein-coupled receptor protein gene may be selected as preferred target regions, though any other region may be selected as a target in G protein coupled receptor protein genes.

Any DNA can be used as the DNA encoding the partial peptide of the receptor protein of the present invention so long as DNA contains the base sequence encoding the partial peptide of the present invention described above. The DNA may be any of genomic DNA, genomic DNA library, cDNA derived from the cells and tissues described above, cDNA library derived from the cells and tissues described above and synthetic DNA. The vector to be used for the library may be any of bacteriophage, plasmid, cosmid and phagemid. The DNA may also be directly amplified by reverse transcriptase polymerase chain reaction (hereinafter abbreviated as RT-PCR) using mRNA fraction prepared from the cells and tissues described above.

Specifically, the DNA encoding the partial peptide of the present invention may be any DNA having the base sequence shown by SEQ ID NO:38, SEQ ID NO:55 or SEQ ID NO:56, or the base sequence hybridizable to the base sequence represented by SEQ ID NO:38, SEQ ID NO:55 or

followed by incubating at 20 to 40°C, preferably at 30 to 37°C for 1 to 10 minutes, an efficient cell fusion can be carried out.

Various methods can be used for screening of a monoclonal antibody-producing hybridoma. Examples of such methods include a method which comprises adding the supernatant of hybridoma to a solid phase (e.g., microplate) adsorbed with the polypeptide (protein) as an antigen directly or together with a carrier, adding an anti-immunoglobulin antibody (where mouse cells are used for the cell fusion, anti-mouse immunoglobulin antibody is used) labeled with a radioactive substance or an enzyme or Protein A and detecting the monoclonal antibody bound to the solid phase, and a method which comprises adding the supernatant of hybridoma to a solid phase adsorbed with an anti-immunoglobulin antibody or Protein A, adding the polypeptide labeled with a radioactive substance or an enzyme and detecting the monoclonal antibody bound to the solid phase.

The monoclonal antibody can be selected according to publicly known methods or their modifications. In general, the selection can be effected in a medium for animal cells supplemented with HAT (hypoxanthine, aminopterin and thymidine). Any selection and growth medium can be employed as far as the hybridoma can grow there. For example, RPMI 1640 medium containing 1% to 20%, preferably 10% to 20% fetal bovine serum, GIT medium (Wako Pure Chemical Industries, Ltd.) containing 1% to 10% fetal bovine serum, a serum free medium for cultivation of a hybridoma (SFM-101, Nissui Seiyaku Co., Ltd.) and the like can be used for the selection and growth medium. The cultivation is carried out generally at 20°C to 40°C, preferably at 37°C, for about 5 days to about 3 weeks, preferably 1 to 2 weeks, normally in 5% CO₂. The antibody titer of the culture supernatant

of a hybridoma can be determined as in the assay for the antibody titer in antisera described above.

(b) Purification of monoclonal antibody

Separation and purification of a monoclonal antibody can be carried out by publicly known methods, such as separation and purification of immunoglobulins (for example, salting-out, alcohol precipitation, isoelectric point precipitation, electrophoresis, adsorption and desorption with ion exchangers (e.g., DEAE), ultracentrifugation, gel filtration, or a specific purification method which comprises collecting only an antibody with an activated adsorbent such as an antigen-binding solid phase, Protein A or Protein G and dissociating the binding to obtain the antibody.

[Preparation of polyclonal antibody]

The polyclonal antibody of the present invention can be manufactured by publicly known methods or modifications thereof. For example, a warm-blooded animal is immunized with an immunogen (polypeptide antigen) per se, or a complex of immunogen and a carrier protein is formed and a warm-blooded animal is immunized with the complex in a manner similar to the method described above for the manufacture of monoclonal antibodies. The product containing the antibody to the polypeptide of the present invention is collected from the immunized animal followed by separation and purification of the antibody.

In the complex of immunogen and carrier protein used to immunize a warm-blooded animal, the type of carrier protein and the mixing ratio of carrier to hapten may be any type and in any ratio, as long as the antibody is efficiently produced to the hapten immunized by crosslinking to the carrier. For example, bovine serum albumin, bovine thyroglobulin or

hemocyanin is coupled to hapten in a carrier-to-hapten weight ratio of approximately 0.1 to 20, preferably about 1 to about 5.

5 A variety of condensation agents can be used for the coupling of carrier to hapten. Glutaraldehyde, carbodiimide, maleimide activated ester and activated ester reagents containing thiol group or dithiopyridyl group are used for the coupling.

10 The condensation product is administered to warm-blooded animals either solely or together with carriers or diluents to the site that can produce the antibody by the administration. In order to potentiate the antibody productivity upon the administration, complete Freund's adjuvant or incomplete Freund's adjuvant may
15 be administered. The administration is usually made once every 2 to 6 weeks and 3 to 10 times in total.

The polyclonal antibody can be collected from the blood, ascites, etc., preferably from the blood of warm-blooded animal immunized by the method described
20 above.

The polyclonal antibody titer in antiserum can be assayed by the same procedure as that for the determination of serum antibody titer described above. The separation and purification of the polyclonal
25 antibody can be carried out, following the method for the separation and purification of immunoglobulins performed as in the separation and purification of monoclonal antibodies described hereinabove.

The antisense DNA having a complementary or
30 substantial complementary base sequence to the DNA coding for the polypeptide of the present invention or its partial peptide or the DNA coding for the receptor protein of the present invention or its partial peptide (hereinafter these DNAs are collectively referred to as
35 the DNA of the present invention in the following

description of antisense DNA) can be any antisense DNA so long as it possesses a base sequence complementary or substantially complementary to that of the DNA of the present invention and capable of suppressing
5 expression of the DNA.

The base sequence substantially complementary to the DNA of the present invention may, for example, be a base sequence having at least about 70% homology, preferably at least about 80% homology, more preferably
10 at least about 90% homology and most preferably at least about 95% homology, to the full-length base sequence or partial base sequence of the base sequence complementary to the DNA of the present invention (i.e., complementary strand to the DNA of the present
15 invention). In the entire base sequence of the complementary strand to the DNA of the present invention, an antisense DNA having at least about 70% homology, preferably at least about 80% homology, more preferably at least about 90% homology and most
20 preferably at least about 95% homology, to the complementary strand of the base sequence which encodes the N-terminal region of the polypeptide of the present invention or the receptor protein of the present invention (e.g., the base sequence around the
25 initiation codon). These antisense DNAs can be synthesized using a publicly known DNA synthesizer, etc.

Hereinafter the utilities of the following substances (1) through (3) are described: (1) the polypeptide of the present invention, its amides or
30 esters, or its partial peptide or its amides or esters, or salts thereof (hereinafter sometimes merely referred to as the polypeptide of the present invention); (2) the receptor protein of the present invention or its salts, or its partial peptide or its amides or esters
35 or salts thereof (hereinafter sometimes merely referred

to as the receptor protein of the present invention);
and (3) DNA encoding the polypeptide of the present
invention or its partial peptide or the receptor
protein of the polypeptide or its partial peptide
5 (hereinafter sometimes merely referred to as the DNA of
the present invention), antibodies to the polypeptide
or the present invention, its amides or esters, or its
partial peptide or its amides or esters, or salts
thereof (hereinafter sometimes merely referred to as
10 the antibody of the present invention) and the
antisense DNA.

(1) Therapeutic and prophylactic agent for the diseases
with which the polypeptide of the present invention or
the receptor protein of the present invention is
15 associated

Since the polypeptide of the present invention has
a cell stimulating activity to the receptor protein of
the present invention, any abnormality or deficiency in
the DNA encoding the polypeptide of the present
20 invention or any abnormality or deficiency in the
receptor protein of the present invention would cause a
variety of diseases such as hypertension, autoimmune
disease, heart failure, cataract, glaucoma, acute
bacterial meningitis, acute myocardial infarction,
25 acute pancreatitis, acute viral encephalitis, adult
respiratory distress syndrome, alcoholic hepatitis,
Alzheimer's disease, asthma, arteriosclerosis, atopic
dermatitis, bacterial pneumonia, bladder cancer,
fracture, breast cancer, bulimia, polyphagia, burn
30 healing, uterine cervical cancer, chronic lymphocytic
leukemia, chronic myelogenous leukemia, chronic
pancreatitis, liver cirrhosis, cancer of the colon and
rectum (colon cancer, rectal cancer), Crohn's disease,
dementia, diabetic complications, diabetic nephropathy,
35 diabetic neuropathy, diabetic retinopathy, gastritis,

healing organ transplantation, burn, wound, alopecia, etc.

(17) These substances of the present invention are also useful as analgesics for suppression or
5 alleviation of chronic or acute pains (pains accompanied by, e.g., postoperative pain, inflammatory pain, toothache, bone disease (e.g., arthritis, rheumatoid, osteoporosis, etc.)).

When a patient has a reduced level of, or
10 deficient in the polypeptide of the present invention or the receptor protein of the present invention in his or her body, the DNA of the present invention can provide its role sufficiently or properly for the patient, (a) by administering the DNA of the present
15 invention to the patient to express the polypeptide of the present invention or the receptor protein of the present invention in vivo, (b) by inserting the DNA of the present invention into a cell, expressing the polypeptide of the present invention or the receptor
20 protein of the present invention and then transplanting the cell to the patient, or (c) by administering the polypeptide of the present invention or the receptor protein of the present invention to the patient.

Where the DNA of the present invention is used as
25 the prophylactic/therapeutic agents described above, the DNA per se is administered directly to human or other warm-blooded animal; alternatively, the DNA is inserted into an appropriate vector such as retrovirus vector, adenovirus vector, adenovirus-associated virus
30 vector, etc. and then administered to human or other warm-blooded animal in a conventional manner. The DNA of the present invention may also be administered as naked DNA, or with adjuvants to assist its uptake by gene gun or through a catheter such as a catheter with
35 a hydrogel.

Since the thus obtained pharmaceutical preparation is safe and low toxic, the preparation can be administered to human or other warm-blooded animal (e.g., rat, mouse, guinea pig, rabbit, chicken, sheep, swine, bovine, horse, cat, dog, monkey, etc.).

The dose of the polypeptide of the present invention or the receptor protein of the present invention varies depending on target disease, subject to be administered, route for administration, etc.; for example, in oral administration for the treatment of nerve disease, the dose is normally about 0.1 mg to about 100 mg, preferably about 1.0 to about 50 mg, and more preferably about 1.0 to about 20 mg per day for adult (as 60 kg body weight). In parenteral administration, the single dose varies depending on subject to be administered, target disease, etc. but it is advantageous for the treatment of nerve disease to administer the active ingredient intravenously at a daily dose of about 0.01 to about 30 mg, preferably about 0.1 to about 20 mg, and more preferably about 0.1 to about 10 mg for adult (as 60 kg body weight). For other animal species, the corresponding dose as converted per 60 kg body weight can be administered.

(2) Screening of drug candidate compounds for disease

Because of the cell stimulating activity or the like by the polypeptide of the present invention to the receptor protein of the present invention, a compound that accelerates or inhibits the functions (e.g., the cell stimulating activity, etc.) of the polypeptide of the present invention or the receptor protein of the present invention, or its salts (these compounds are also referred to as a compound that alter the binding property between the polypeptide of the present invention and the receptor protein of the present invention, or its salts; hereinafter the same) can be

tuberculosis, spinal injury, bone fracture, hepatic insufficiency, pneumonia, alcoholic hepatitis, hepatitis A, hepatitis B, hepatitis C, AIDS infectious disease, human papilloma virus infectious disease, influenza infectious disease, cancer metastasis, multiple myeloma, osteomalacia, osteoporosis, Behcet's disease of bone, nephritis, renal insufficiency, sepsis, septic shock, hypercalcemia, hypercholesterolemia, hyperglyceridemia, hyperlipemia, systemic lupus erythematosus, transient cerebral ischemia, alcoholic hepatitis, etc.

(16) The compound or its salts are also used for healing organ transplantation, burn, wound, alopecia, etc.

(17) The compound or its salts are also useful as analgesics for suppression or alleviation of chronic or acute pains (pains accompanied by, e.g., postoperative pain, inflammatory pain, toothache, bone disease (e.g., arthritis, rheumatoid, osteoporosis, etc.)).

Therefore, the polypeptide of the present invention or the receptor protein of the present invention is useful as reagents for screening the compound or its salts that accelerate or inhibit the functions of the polypeptide of the present invention or the receptor protein of the present invention.

That is, the present invention provides:

(1) a method for screening the compound or its salts that accelerate the functions (e.g., a cell stimulating activity, a somatostatin secretion regulating activity, etc.) of the polypeptide of the present invention, its amides or esters, or salts thereof, the partial peptide, its amides or esters, or salts thereof (hereinafter sometimes merely referred to as the accelerator), or the compound or its salts that inhibit the functions of the polypeptide of the present

(e.g., a cell stimulating activity, a somatostatin secretion regulating activity, etc.) of the polypeptide of the present invention, its amides or esters, or salts thereof, the partial peptide, its amides or esters, or salts thereof, or the receptor protein of the present invention or its salts, or the partial peptide, its amides or esters, or salts thereof (hereinafter sometimes merely referred to as the inhibitor), which comprises using the polypeptide of the present invention, its amides or esters, or salts thereof, or the partial peptide, its amides or esters, or salts thereof, or the receptor protein of the present invention or its salts, or the partial peptide, its amides or esters, or salts thereof (specifically the protein containing the same or substantially the same amino acid sequence as the amino acid sequence shown by SEQ ID NO:37, or salts thereof, or the partial peptide, its amides or esters, or salts thereof).

The present invention further provides:

(3) a method for screening the compound or its salts that accelerate the functions (e.g., a cell stimulating activity, a somatostatin secretion regulating activity, etc.) of the polypeptide of the present invention, its amides or esters, or salts thereof, the partial peptide, its amides or esters, or salts thereof, or the receptor protein of the present invention or its salts, or the partial peptide, its amides or esters, or salts thereof (hereinafter sometimes merely referred to as the accelerator), or, the compound or its salts that inhibits the functions (e.g., a cell stimulating activity, a somatostatin secretion regulating activity, etc.) of the polypeptide of the present invention, its amides or esters, or salts thereof, the partial peptide, its amides or esters, or salts thereof, or the receptor protein of

the present invention or its salts, or the partial peptide, its amides or esters, or salts thereof (hereinafter sometimes merely referred to as the inhibitor), which comprises measuring

- 5 (i) the activity of the polypeptide of the present invention, its amides or esters, or salts thereof, or the partial peptide, its amides or esters, or salts thereof, when the receptor protein of the present invention or its salts, or the partial peptide, its amides or esters, or salts thereof (specifically the protein containing the same or substantially the same amino acid sequence as the amino acid sequence shown by SEQ ID NO:37, or salts thereof, or the partial peptide, its amides or esters, or salts thereof) is brought into
- 10 contact with the polypeptide of the present invention, its amides or esters, or salts thereof, or the partial peptide, its amides or esters, or salts thereof, and
- 15 (ii) the activity of the polypeptide of the present invention, its amides or esters, or salts thereof, or the partial peptide, its amides or esters, or salts thereof, when the receptor protein of the present invention or its salts, or the partial peptide, its amides or esters, or salts thereof (specifically the protein containing the same or substantially the same amino acid sequence as the amino acid sequence shown by SEQ ID NO:37, or salts thereof, or the partial peptide, its amides or esters, or salts thereof) and a test compound are brought into contact with the polypeptide of the present invention, its amides or
- 20 esters, or salts thereof, or the partial peptide, its amides or esters, or salts thereof;
- 25 and comparing the activities;
- 30 and the like.

Specifically, the screening method described above

35 is characterized by measuring the cell stimulating

activities of the polypeptide of the present invention and a test compound or the binding amount of the polypeptide of the present invention and a test compound to the receptor protein of the present invention, in the cases (i) and (ii), and comparing these activities.

The activities of the polypeptide in the present invention such as the cell stimulating activity, etc. can be measured in accordance with publicly known methods, for example, Dockray, G.J., et al., Nature, 305, 328-330, 1983, Fukusumi, S., et al., Biochem. Biophys. Res. Commun., 232, 157-163, 1997, Hinuma, S., et al., Nature, 393, 272-276, 1998, Tatemoto, K., et al., Biochem. Biophys. Res. Commun., 251, 471-476, 1998, etc., or modifications thereof.

The binding amount of the polypeptide of the present invention and a test compound to the receptor protein of the present invention can be measured by a modification of the methods for "determination of a ligand (agonist) to the receptor protein of the present invention" which will be described hereinafter.

Examples of such a test compound are a peptide, a protein, a non-peptide compound, a synthetic compound, a fermentation product, a cell extract, a plant extract, an animal tissue extract and the like. These compounds may be novel compounds or publicly known compounds.

To perform the screening method described above, the polypeptide of the present invention is suspended in a buffer suitable for screening to prepare a specimen of the polypeptide of the present invention. Any buffer having pH of approximately 4 to 10 (desirably pH of approximately 6 to 8) such as a phosphate buffer, Tris-hydrochloride buffer, etc. may be used so long as it does not interfere the reaction

between the polypeptide of the present invention and the receptor protein of the present invention.

For example, when a test compound increases the cell stimulating activity, etc. in (ii) described above by at least about 20%, preferably at least 30%, more preferably at least about 50% as compared to the case of (i) above, the test compound can be selected to be a compound that accelerates the cell stimulating activity, etc. of the polypeptide of the present invention. On the other hand, a test compound can be selected to be a compound that inhibits the cell stimulating activity, etc. of the polypeptide of the present invention, when the test compound inhibits the cell stimulating activity, etc. in (ii) described above by at least about 20%, preferably at least 30%, more preferably at least about 50% as compared to the case of (i) above.

It is desirable, before conducting these tests, to examine the binding ability of a test compound to the receptor protein of the present invention to see if the test compound is capable of binding to the receptor protein of the present invention, which is effected by the methods (1) to (3) later described for the "determination of ligand (agonist) to the receptor protein of the present invention".

As an index that the test compound described above is judged to be the compound or its salts that accelerate or inhibit the activities of the polypeptide of the present invention, there is such an activity that inhibit the binding between the receptor protein of the present invention and the labeled polypeptide of the present invention or its partial peptide. According to the binding test system described, e.g., in Hosoya, M. et al., Biochem. Biophys. Res. Commun., 194 (1), 133-134, 1993, a test compound that inhibits the binding of the labeled compound by at least 10% in a

concentration of 1×10^{-2} M or less is highly likely to be the compound or salts that accelerate or inhibit the activities of the polypeptide of the present invention. However, since the binding inhibition activity is a relative value based on the binding of the labeled compound, the activity is not essential for judging the test compound to be a compound or salts that accelerate or inhibit the activities of the polypeptide of the present invention.

10 The kit for screening of the present invention comprises the polypeptide of the present invention, its amides or esters, or salts thereof, the partial peptide, its amides or esters, or salts thereof. Preferably, the kit for screening of the present invention further
15 comprises the receptor to the polypeptide of the present invention, its amides or esters, or salts thereof, the partial peptide, its amides or esters, or salts thereof, that is, the receptor protein of the present invention or its salts, the partial peptide,
20 its amides or esters, or salts thereof (specifically, the protein containing the same or substantially the same amino acid sequence as that shown by SEQ ID NO:37).

Examples of the screening kit according to the present invention include the following:

25 1. Reagent for screening

(1) Buffers for assay and washing

Hanks' Balanced Salt Solution (manufactured by Gibco) supplemented with 0.05% of bovine serum albumin (manufactured by Sigma).

30 The buffers may be sterilized by filtration through a membrane filter with a $0.45 \mu\text{m}$ pore size and stored at 4°C , or may be prepared at use.

(2) A receptor preparation

CHO cells in which the receptor protein of the present invention is expressed are subcultured at $5 \times$

10^5 cells/well on a 12-well plate followed by culturing at 37°C under a 5% CO₂ and 95% air for 2 days.

(3) Labeled ligand

The polypeptide of the present invention, its amides or esters, or the partial peptide, its amides or esters are labeled with commercially available [³H], [¹²⁵I], [¹⁴C], [³⁵S], etc. The product in the form of an aqueous solution is stored at 4°C or at -20°C, which will be diluted at use to 1 μM with a buffer for the assay.

(4) Standard ligand solution

The polypeptide of the present invention, its amides or esters, or the partial peptide, its amides or esters, are dissolved in PBS containing 0.1% of bovine serum albumin (manufactured by Sigma) to make the volume 1 mM and then stored at -20°C.

2. Method for assay

(1) CHO cells are cultured in a 12-well tissue culture plate to express the receptor protein of the present invention. After washing the CHO cells twice with 1 ml of buffer for the assay, 490 μl of the buffer for assay is added to each well.

(2) After 5 μl of a test compound solution of 10^{-3} to 10^{-10} M is added, 5 μl of a labeled ligand is added to the system followed by incubating at room temperature for an hour. To determine the amount of the non-specific binding, 5 μl of the ligand of 10^{-3} M is added to the system, instead of the test compound.

(3) The reaction mixture is removed from the well, which is washed three times with 1 ml each of the buffer for assay. The labeled ligand bound to the cells is dissolved in 0.2N NaOH-1% SDS and mixed with 4 ml of a liquid scintillator A (manufactured by Wako Pure Chemical).

(4) Radioactivity is measured using a liquid scintillation counter (manufactured by Beckman) and PMB (percent of the maximum binding) is calculated in accordance with the following equation:

5

$$\text{PMB} = [(B - \text{NSB}) / (B_0 - \text{NSB})] \times 100$$

wherein:

10 PMB: percent of the maximum binding
 B: value when a sample is added
 NSB: non-specific binding
 B₀: maximum binding

15 The compound or a salt thereof obtainable by the screening method or by the screening kit of the present invention is the compound selected from the test compounds described above, such as peptides, proteins, non-peptide compounds, synthetic compounds, fermentation products, cell extracts, plant extracts, 20 animal tissue extracts, plasma, etc. and the compound that accelerates or inhibits the functions (e.g., a cell stimulating activity, a somatostatin secretion regulating activity, etc.) of the polypeptide of the present invention.

25 As the salts of the compound, there may be employed similar salts to those of the polypeptide of the present invention described above.

When the compound or salts thereof obtainable by the screening method or the screening kit of the present invention are used as the therapeutic and 30 prophylactic agents described above, a conventional means may be applied to making pharmaceutical preparations. For example, the compound or its salts may be prepared into tablets, capsules, elixirs, 35 microcapsules, sterile solutions, suspensions, etc.

Since the thus obtained preparation is safe and low toxic, it can be administered to human or warm-blooded animal (e.g., mouse, rat, rabbit, sheep, swine, bovine, horse, chicken, cat, dog, monkey, etc.).

5 The dose of the compound or salts thereof varies depending on activity, target disease, subject to be administered, method for administration, etc.; for example, in oral administration of the compound that accelerates the functions of the polypeptide of the
10 present invention, the dose is normally about 0.1 to about 100 mg, preferably about 1.0 to about 50 mg, more preferably about 1.0 to about 20 mg per day for adult (as 60 kg body weight). In parenteral administration, the single dose varies depending on subject to be
15 administered, target disease, etc. but it is advantageous to administer, for example, the compound that accelerates the functions of the polypeptide of the present invention intravenously at a daily dose of about 0.01 to about 30 mg, preferably about 0.1 to
20 about 20 mg, more preferably about 0.1 to about 10 mg for adult (as 60 kg body weight). For other animal species, the corresponding dose as converted per 60 kg weight can be administered.

Turning to the compound that inhibits the
25 functions of the polypeptide of the present invention when it is orally administered, the dose is normally about 0.1 to about 100 mg, preferably about 1.0 to about 50 mg, more preferably about 1.0 to about 20 mg per day for adult (as 60 kg body weight). In parenteral
30 administration, the single dose varies depending on subject to be administered, target disease, etc. When the compound that inhibits the functions of the polypeptide of the present invention is administered to adult (as 60 kg body weight) generally in the form of
35 injection, it is advantageous to administer the

compound intravenously at a daily dose of about 0.01 to about 30 mg, preferably about 0.1 to about 20 mg, more preferably about 0.1 to about 10 mg. For other animal species, the corresponding dose as converted per 60 kg weight can be administered.

(3) Quantification for the polypeptide of the present invention, its amides or esters, or salts thereof, the partial peptide, its amides or esters, or salts thereof, and the receptor protein of the present invention or salts thereof, or the partial peptide, its amides or esters, or salts thereof:

The antibody to the polypeptide of the present invention or the receptor protein of the present invention (hereinafter sometimes merely referred to as the antibody of the present invention) is capable of specifically recognizing the polypeptide of the present invention or the receptor protein of the present invention and thus, can be used for a quantification of the polypeptide of the present invention or the receptor protein of the present invention in a test sample fluid, in particular, for a quantification by sandwich immunoassay.

That is, the present invention provides:

(i) a method for quantification of the polypeptide of the present invention or the receptor protein of the present invention in a test sample fluid, which comprises competitively reacting the antibody of the present invention, a test sample fluid and the labeled polypeptide of the present invention or the labeled receptor protein of the present invention, and measuring the ratio of the labeled polypeptide of the present invention or the labeled receptor protein of the present invention bound to said antibody; and,

(ii) a method for quantification of the polypeptide of the present invention or the receptor

whereby the amount of the polypeptide of the present
 invention or the receptor protein of the present
 invention in the test sample fluid can be quantified.
 The first and second reactions may be carried out in a
 5 reversed order, simultaneously or sequentially with an
 interval. The type of the labeling agent and the method
 for immobilization may be the same as those described
 hereinabove. In the immunoassay by the sandwich method,
 it is not always necessary that the antibody used for
 10 the labeled antibody and for the solid phase should be
 one type or one species but a mixture of two or more
 antibodies may also be used for the purpose of
 improving the measurement sensitivity, etc.

In the method for assaying the polypeptide of the
 15 present invention or the receptor protein of the
 present invention by the sandwich method according to
 the present invention, preferred monoclonal antibodies
 of the present invention used for the first and the
 second reactions are antibodies, which binding sites to
 20 the polypeptide of the present invention or the
 receptor protein of the present invention are different
 from one another. Thus, the antibodies used in the
 first and the second reactions are those wherein, when
 the antibody used in the second reaction recognizes the
 25 C-terminal region of the polypeptide of the present
 invention or the receptor protein, the antibody
 recognizing the site other than the C-terminal regions,
 e.g., recognizing the N-terminal region, is preferably
 used in the first reaction.

30 The monoclonal antibody of the present invention
 may be used in an assay system other than the sandwich
 method, such as a competitive method, an immunometric
 method and a nephrometry.

In the competitive method, an antigen in a test
 35 sample fluid and a labeled antigen are competitively

reacted with an antibody, then the unreacted labeled antigen (F) and the labeled antigen bound to the antibody (B) are separated (i.e., B/F separation) and the labeled amount of either B or F is measured to
5 determine the amount of the antigen in the test sample fluid. In the reactions for such a method, there are a liquid phase method in which a soluble antibody is used as the antibody and the B/F separation is effected by polyethylene glycol while a second antibody to the
10 antibody is used, and a solid phase method in which an immobilized antibody is used as the first antibody or a soluble antibody is used as the first antibody while an immobilized antibody is used as the second antibody.

In the immunometric method, an antigen in a test
15 sample fluid and an immobilized antigen are competitively reacted with a given amount of a labeled antibody followed by separating the solid phase from the liquid phase; or an antigen in a test sample fluid and an excess amount of labeled antibody are reacted,
20 then an immobilized antigen is added to bind an unreacted labeled antibody to the solid phase and the solid phase is separated from the liquid phase. Thereafter, the labeled amount of any of the phases is measured to determine the antigen amount in the test
25 sample fluid.

In the nephrometry, the amount of insoluble sediment, which is produced as a result of the antigen-antibody reaction in a gel or in a solution, is measured. Even when the amount of an antigen in a test
30 sample fluid is small and only a small amount of the sediment is obtained, a laser nephrometry utilizing laser scattering can be suitably used.

In applying each of those immunoassays to the assay method for the present invention, any special
35 conditions or operations are not required to set forth.

The assay system for the polypeptide of the present invention may be constructed in addition to conditions or operations conventionally used for each of the methods, taking the technical consideration of one skilled in the art into account consideration. For the details of such conventional technical means, a variety of reviews, reference books, etc. may be referred to (for example, Hiroshi Irie (ed.): "Radioimmunoassay" (published by Kodansha, 1974); Hiroshi Irie (ed.): "Radioimmunoassay; Second Series" (published by Kodansha, 1979); Eiji Ishikawa, et al. (ed.): "Enzyme Immunoassay" (published by Igaku Shoin, 1978); Eiji Ishikawa, et al. (ed.): "Enzyme Immunoassay" (Second Edition) (published by Igaku Shoin, 1982); Eiji Ishikawa, et al. (ed.): "Enzyme Immunoassay" (Third Edition) (published by Igaku Shoin, 1987); "Methods in Enzymology" Vol. 70 (Immunochemical Techniques (Part A)); *ibid.*, Vol. 73 (Immunochemical Techniques (Part B)); *ibid.*, Vol. 74 (Immunochemical Techniques (Part C)); *ibid.*, Vol. 84 (Immunochemical Techniques (Part D: Selected Immunoassays)); *ibid.*, Vol. 92 (Immunochemical Techniques (Part E: Monoclonal Antibodies and General Immunoassay Methods)); *ibid.*, Vol. 121 (Immunochemical Techniques (Part I: Hybridoma Technology and Monoclonal Antibodies)) (published by Academic Press); etc.)

As described above, the polypeptide of the present invention or the receptor protein of the present invention can be quantified with high sensitivity, using the antibody of the present invention.

Furthermore, when a decrease or increase in level of the polypeptide of the present invention or the receptor protein of the present invention is detected by quantifying the level of the polypeptide of the present invention or the receptor protein using the antibody of the present invention, it can be diagnosed

that the following diseases are involved or it is highly likely to suffer from these disease in the future. Examples of such diseases are hypertension, autoimmune disease, heart failure, cataract, glaucoma, acute bacterial meningitis, acute myocardial infarction, acute pancreatitis, acute viral encephalitis, adult respiratory distress syndrome, alcoholic hepatitis, Alzheimer's disease, asthma, arteriosclerosis, atopic dermatitis, bacterial pneumonia, bladder cancer, fracture, breast cancer, bulimia, polyphagia, burn healing, uterine cervical cancer, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic pancreatitis, liver cirrhosis, cancer of the colon and rectum (colon cancer, rectal cancer), Crohn's disease, dementia, diabetic complications, diabetic nephropathy, diabetic neuropathy, diabetic retinopathy, gastritis, Helicobacter pylori bacterial infectious disease, hepatic insufficiency, hepatitis A, hepatitis B, hepatitis C, hepatitis, herpes simplex virus infectious disease, varicellazoster virus infectious disease, Hodgkin's disease, AIDS infectious disease, human papilloma virus infectious disease, hypercalcemia, hypercholesterolemia, hyperglyceridemia, hyperlipemia, infectious disease, influenza infectious disease, insulin dependent diabetes mellitus (type I), invasive staphylococcal infectious disease, malignant melanoma, cancer metastasis, multiple myeloma, allergic rhinitis, nephritis, non-Hodgkin's lymphoma, insulin-independent diabetes mellitus (type II), non-small cell lung cancer, organ transplantation, arthroseitis, osteomalacia, osteopenia, osteoporosis, ovarian cancer, Behcet's disease of bone, peptic ulcer, peripheral vessel disease, prostatic cancer, reflux esophagitis, renal insufficiency, rheumatoid arthritis, schizophrenia, sepsis, septic shock, severe systemic fungal infectious

disease, small cell lung cancer, spinal injury, stomach cancer, systemic lupus erythematosus, transient cerebral ischemia, tuberculosis, cardiac valve failure, vascular/multiple infarction dementia, wound healing,
 5 insomnia, arthritis, pituitary hormone secretion disorder, pollakiuria, uremia, neurodegenerative disease, etc.

Where a decrease or increase in the level of the polypeptide of the present invention or the receptor
 10 protein of the present invention is detected, it can be diagnosed as well that a disease such as macular edema cystoid or the like is involved or it is highly likely to suffer from such a disease in the future.

Moreover, since the polypeptide of the present
 15 invention, the receptor protein of the present invention and the DNA of the present invention are associated with secretion control of somatostatin, it can be diagnosed that the following diseases are involved or there is a high possibility to suffer from
 20 these diseases in the future, when a decreased or increased level of the polypeptide of the present invention or the receptor protein of the present invention is detected. Examples of these diseases are:

(1) acromegaly, TSH-producing tumor, non-secretory
 25 (non-functional) pituitary tumor, ectopic ACTH (adrenocorticotropine)-producing tumor, medullary thyroid cancer, VIP-producing tumor, glucagon-producing tumor, gastrin-producing tumor, insulinoma, carcinoid tumor;

(2) insulin-dependent or insulin-independent
 30 diabetes mellitus, or various diseases associated with the diabetes, i.e., diabetic complications (e.g., diabetic retinopathy, diabetic nephropathy, diabetic neuropathy, Down's syndrome, orthostatic hypotension,
 35 etc.);

hyperglyceridemia, hyperlipemia, systemic lupus erythematosus, transient cerebral ischemia, alcoholic hepatitis;

(16) burn, wound, alopecia;

5 (17) chronic or acute pains (pains accompanied by, e.g., postoperative pain, inflammatory pain, toothache, bone disease (e.g., arthritis, rheumatoid, osteoporosis, etc.)).

The antibody of the present invention can be
10 employed for detecting the polypeptide of the present invention or the receptor protein of the present invention which may be present in a test sample fluid such as a body fluid, a tissue, etc. The antibody can also be used for preparation of an antibody column for
15 purification of the polypeptide of the present invention or the receptor protein of the present invention, detection of the receptor protein of the present invention in the fractions upon purification, and analysis of the behavior of the polypeptide of the
20 present invention or the receptor protein of the present invention in the cells under investigation.

(4) Gene diagnostic agent

By using the DNA of the present invention, e.g., as a probe, an abnormality (gene abnormality) of the
25 DNA or mRNA coding for the polypeptide of the present invention or the receptor protein of the present invention in human or warm-blooded animal (e.g., rat, mouse, guy pig, rabbit, chicken, sheep, swine, bovine, horse, cat, dog, monkey, etc.) can be detected.
30 Therefore, the DNA of the present invention is useful as a gene diagnostic agent for the damage to the DNA or mRNA, its mutation, or its decreased expression, or increased expression or overexpression of the DNA or mRNA.

cancer metastasis, multiple myeloma, allergic rhinitis, nephritis, non-Hodgkin's lymphoma, insulin-independent diabetes mellitus (type II), non-small cell lung cancer, organ transplantation, arthroseitis, osteomalacia, osteopenia, osteoporosis, ovarian cancer, Behcet's disease of bone, peptic ulcer, peripheral vessel disease, prostatic cancer, reflux esophagitis, renal insufficiency, rheumatoid arthritis, schizophrenia, sepsis, septic shock, severe systemic fungal infectious disease, small cell lung cancer, spinal injury, stomach cancer, systemic lupus erythematosus, transient cerebral ischemia, tuberculosis, cardiac valve failure, vascular/multiple infarction dementia, wound healing, insomnia, arthritis, pituitary hormone secretion disorder, pollakiuria, uremia, neurodegenerative disease, etc.

In case that a decreased expression or overexpression is detected by the Northern hybridization, it can also be diagnosed that a disease such as macular edema cystoid or the like is involved or it is highly likely to suffer from such a disease in the future.

In addition, since the polypeptide of the present invention, the receptor protein of the present invention and the DNA of the present invention are associated with secretion control of somatostatin, the decrease in expression or overexpression detected by the Northern hybridization results in such a diagnosis that the following diseases are involved or there is a high possibility to suffer from these diseases in the future. Examples of the diseases are:

(1) acromegaly, TSH-producing tumor, non-secretory (non-functional) pituitary tumor, ectopic ACTH (adrenocorticotropine)-producing tumor, medullary thyroid cancer, VIP-producing tumor, glucagon-producing

- (9) tumor or cancer (e.g., thyroid cancer, colon cancer, breast cancer, prostatic cancer, small cell lung cancer, non-small cell lung cancer, pancreatic cancer, gastric cancer, bile duct cancer, liver cancer, bladder cancer, ovary cancer, melanoma, osteosarcoma, chondrosarcoma, malignant pheochromocytoma, neuroblastoma, brain tumor, thymoma, kidney cancer, etc.), leukemia (e.g., leukemia/chronic lymphoid leukemia of basophil leukocyte, chronic myeloid leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, etc.), these agents may also be used alone or in combination with other carcinostatic agents (e.g., tamoxifen, LHRH agonist, LHRH antagonist, interferon- α , β and γ , interleukin-2, etc.);
- (10) hypertrophic cardiomyopathy, arteriosclerosis, valvular disease, myocardial infarction (especially myocardial infarction after percutaneous transluminal angioplasty) or regeneration of blood vessels;
- (11) hemorrhage in esophageal venous cancer, cirrhosis or peripheral vessel disease;
- (12) disease accompanied by regulation of secretion of physiologically active substances acting on the immune system, such as systemic or regional inflammation (e.g., multiple arthritis, rheumatoid arthritis, psoriasis, sunburn, eczema, allergy (e.g., asthma, atopic dermatitis, allergic rhinitis, etc.);
- (13) dementia (e.g., Alzheimer's disease, Alzheimer's senile dementia, vascular/multiple dementia, etc.), schizophrenia, epilepsy, depression, general anxiety disorder, sleeping disorder, multiple sclerosis;
- (14) eye disease (e.g., glaucoma, etc.);
- (15) acute bacterial meningitis, acute viral encephalitis, adult respiratory distress syndrome, bacterial pneumonia, severe systemic fungal infectious

disease, tuberculosis, spinal injury, bone fracture,
 hepatic insufficiency, pneumonia, alcoholic hepatitis,
 hepatitis A, hepatitis B, hepatitis C, AIDS infectious
 disease, human papilloma virus infectious disease,
 5 influenza infectious disease, cancer metastasis,
 multiple myeloma, osteomalacia, osteoporosis, Behcet's
 disease of bone, nephritis, renal insufficiency, sepsis,
 septic shock, hypercalcemia, hypercholesterolemia,
 hyperglyceridemia, hyperlipemia, systemic lupus
 10 erythematosus, transient cerebral ischemia, alcoholic
 hepatitis;

(16) burn, wound, alopecia;

(17) chronic or acute pains (pains accompanied by,
 e.g., postoperative pain, inflammatory pain, toothache,
 15 bone disease (e.g., arthritis, rheumatoid, osteoporosis,
 etc.)).

(5) Pharmaceutical composition comprising antisense DNA

Antisense DNA that binds to the DNA of the present
 invention complementarily to inhibit expression of the
 20 DNA can be used as the agent for the
 treatment/prevention of diseases such as hypertension,
 autoimmune disease, heart failure, cataract, glaucoma,
 acute bacterial meningitis, acute myocardial infarction,
 acute pancreatitis, acute viral encephalitis, adult
 25 respiratory distress syndrome, alcoholic hepatitis,
 Alzheimer's disease, asthma, arteriosclerosis, atopic
 dermatitis, bacterial pneumonia, bladder cancer,
 fracture, breast cancer, bulimia, polyphagia, burn
 healing, uterine cervical cancer, chronic lymphocytic
 30 leukemia, chronic myelogenous leukemia, chronic
 pancreatitis, liver cirrhosis, cancer of the colon and
 rectum (colon cancer, rectal cancer), Crohn's disease,
 dementia, diabetic complications, diabetic nephropathy,
 diabetic neuropathy, diabetic retinopathy, gastritis,
 35 Helicobacter pylori bacterial infectious disease,

hepatic insufficiency, hepatitis A, hepatitis B, hepatitis C, hepatitis, herpes simplex virus infectious disease, varicellazoster virus infectious disease, Hodgkin's disease, AIDS infectious disease, human

5 papilloma virus infectious disease, hypercalcemia, hypercholesterolemia, hyperglyceridemia, hyperlipemia, infectious disease, influenza infectious disease, insulin dependent diabetes mellitus (type I), invasive staphylococcal infectious disease, malignant melanoma,

10 cancer metastasis, multiple myeloma, allergic rhinitis, nephritis, non-Hodgkin's lymphoma, insulin-independent diabetes mellitus (type II), non-small cell lung cancer, organ transplantation, arthroseitis, osteomalacia, osteopenia, osteoporosis, ovarian cancer, Behcet's

15 disease of bone, peptic ulcer, peripheral vessel disease, prostatic cancer, reflux esophagitis, renal insufficiency, rheumatoid arthritis, schizophrenia, sepsis, septic shock, severe systemic fungal infectious disease, small cell lung cancer, spinal injury, stomach

20 cancer, systemic lupus erythematosus, transient cerebral ischemia, tuberculosis, cardiac valve failure, vascular/multiple infarction dementia, wound healing, insomnia, arthritis, pituitary hormone secretion disorder, pollakiuria, uremia, neurodegenerative

25 disease, etc.

The antisense DNA that binds to the DNA of the present invention and can inhibit expression of the DNA can also be used as the therapeutic/prophylactic agent for macular edema cystoid.

30 In addition, since the polypeptide of the present invention or the receptor protein of the present invention are associated with secretion control of somatostatin, the antisense DNA that binds to the DNA of the present invention and can inhibit expression of

35 the DNA are useful as:

(1) therapeutic agents for tumors such as acromegaly, TSH-producing tumor, non-secretory (non-functional) pituitary tumor, ectopic ACTH (adrenocorticotropine)-producing tumor, medullary thyroid cancer, VIP-producing tumor, glucagon-producing tumor, gastrin-producing tumor, insulinoma, carcinoid tumor, etc.;

(2) therapeutic agents for insulin-dependent or insulin-independent diabetes mellitus, or various diseases associated with the diabetes, i.e., diabetic complications (e.g., diabetic retinopathy, diabetic nephropathy, diabetic neuropathy, Down's syndrome, orthostatic hypotension, etc.);

(3) agents for improving hyperinsulinism or for the treatment of obesity, bulimia, etc. caused by the suppression of appetite;

(4) therapeutic agents for acute pancreatitis, chronic pancreatitis, pancreatic/intestinal fistula, hemorrhagic ulcer, peptic ulcer, gastritis, hyperchylia, reflux esophagitis, etc.;

(5) agents for alleviating various conditions accompanied by *Helicobacter pylori* bacterial infections (e.g., an agent for suppressing accentuated gastrin secretion, etc.);

(6) agents for suppressing secretion of amylase accompanied by endoscopic cholangio pancreatography and for the postoperative treatment in pancreas surgery;

(7) agents for the treatment of diarrhea caused by reduced absorption or accentuated secretion in small intestine or abnormal motility of digestive tract (Short bowel syndrome, etc.), diarrhea caused by drugs in chemotherapy of cancer, etc., diarrhea caused by congenital small intestine atrophy, diarrhea caused by neuro-endocrinal tumor such as VIP-producing tumor, etc., diarrhea caused by AIDS, diarrhea caused by

graft-versus-host reaction accompanied by spinal
transplant, etc., diarrhea caused by diabetes mellitus,
diarrhea caused by blocking nervous plexus in the
abdominal cavity, diarrhea caused by systemic sclerosis,
5 diarrhea caused by eosinophilia, etc.;

(8) agents for the treatment of Dumping syndrome,
hypersensitive colitis, Crohn's disease, inflammatory
bowel disease, etc.;

(9) agents for the treatment of tumor or cancer
10 (e.g., thyroid cancer, colon cancer, breast cancer,
prostatic cancer, small cell lung cancer, non-small
cell lung cancer, pancreatic cancer, gastric cancer,
bile duct cancer, liver cancer, bladder cancer, ovary
cancer, melanoma, osteosarcoma, chondrosarcoma,
15 malignant pheochromocytoma, neuroblastoma, brain tumor,
thymoma, kidney cancer, etc.), leukemia (e.g.,
leukemia/chronic lymphoid leukemia of basophil
leukocyte, chronic myeloid leukemia, Hodgkin's disease,
non-Hodgkin's lymphoma, etc.), these agents may also be
20 used alone or in combination with other carcinostatic
agents (e.g., tamoxifen, LHRH agonist, LHRH antagonist,
interferon- α , β and γ , interleukin-2, etc.);

(10) agents for the prevention and treatment of
hypertrophic cardiomyopathy, arteriosclerosis, valvular
25 disease, myocardial infarction (especially myocardial
infarction after percutaneous transluminal angioplasty)
or regeneration of blood vessels;

(11) agents for the treatment of hemorrhage in
esophageal venous cancer, cirrhosis or peripheral vessel
30 disease;

(12) agents for the treatment of disease
accompanied by regulation of secretion of
physiologically active substances acting on the immune
system, such as systemic or regional inflammation (e.g.,
35 multiple arthritis, rheumatoid arthritis, psoriasis,

sunburn, eczema, allergy (e.g., asthma, atopic dermatitis, allergic rhinitis, etc.), etc.;

(13) agents for the treatment of, for example, dementia (e.g., Alzheimer's disease, Alzheimer's senile dementia, vascular/multiple dementia, etc.),
5 schizophrenia, epilepsy, depression, general anxiety disorder, sleeping disorder, multiple sclerosis, etc.;

(14) agents for the treatment of eye disease (e.g., glaucoma, etc.), etc.;

10 (15) agents for the prevention and treatment of acute bacterial meningitis, acute viral encephalitis, adult respiratory distress syndrome, bacterial pneumonia, severe systemic fungal infectious disease, tuberculosis, spinal injury, bone fracture, hepatic
15 insufficiency, pneumonia, alcoholic hepatitis, hepatitis A, hepatitis B, hepatitis C, AIDS infectious disease, human papilloma virus infectious disease, influenza infectious disease, cancer metastasis, multiple myeloma, osteomalacia, osteoporosis, Behcet's
20 disease of bone, nephritis, renal insufficiency, sepsis, septic shock, hypercalcemia, hypercholesterolemia, hyperglyceridemia, hyperlipemia, systemic lupus erythematosus, transient cerebral ischemia, alcoholic hepatitis, etc.

25 (16) The antisense DNA can also be used for healing organ transplantation, burn, wound, alopecia, etc.

(17) The antisense DNA is also useful as analgesics for suppression or alleviation of chronic or
30 acute pains (pains accompanied by, e.g., postoperative pain, inflammatory pain, toothache, bone disease (e.g., arthritis, rheumatoid, osteoporosis, etc.)).

In the case that the antisense DNA described above is used as the therapeutic/prophylactic agent, the
35 therapeutic/prophylactic agents for various diseases

described above comprising the DNA of the present invention apply similarly to the antisense DNA.

For example, when the antisense DNA is used, the antisense DNA is administered directly, or the
5 antisense DNA is inserted into an appropriate vector such as retrovirus vector, adenovirus vector, adenovirus-associated virus vector, etc. followed by treating in a conventional manner. The antisense DNA may be administered as it stands, or with a
10 physiologically acceptable carrier to assist its uptake by gene gun or through a catheter such as a catheter with a hydrogel.

In addition, the antisense DNA may also be employed as an oligonucleotide probe for diagnosis to
15 examine the presence of the DNA of the present invention in tissues or cells and states of its expression.

(6) Pharmaceutical composition comprising the antibody of the present invention

20 The antibody of the present invention which possesses the effect to neutralize the activities of the polypeptide of the present invention or the receptor peptide of the present invention can be used as drugs for the treatment/prevention of diseases such
25 as hypertension, autoimmune disease, heart failure, cataract, glaucoma, acute bacterial meningitis, acute myocardial infarction, acute pancreatitis, acute viral encephalitis, adult respiratory distress syndrome, alcoholic hepatitis, Alzheimer's disease, asthma,
30 arteriosclerosis, atopic dermatitis, bacterial pneumonia, bladder cancer, fracture, breast cancer, bulimia, polyphagia, burn healing, uterine cervical cancer, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic pancreatitis, liver
35 cirrhosis, cancer of the colon and rectum (colon

cancer/rectal cancer), Crohn's disease, dementia,
diabetic complications, diabetic nephropathy, diabetic
neuropathy, diabetic retinopathy, gastritis,
Helicobacter pylori bacterial infectious disease,
5 hepatic insufficiency, hepatitis A, hepatitis B,
hepatitis C, hepatitis, herpes simplex virus infectious
disease, varicellazoster virus infectious disease,
Hodgkin's disease, AIDS infectious disease, human
papilloma virus infectious disease, hypercalcemia,
10 hypercholesterolemia, hyperglyceridemia, hyperlipemia,
infectious disease, influenza infectious disease,
insulin dependent diabetes mellitus (type I), invasive
staphylococcal infectious disease, malignant melanoma,
cancer metastasis, multiple myeloma, allergic rhinitis,
15 nephritis, non-Hodgkin's lymphoma, insulin-independent
diabetes mellitus (type II), non-small cell lung cancer,
organ transplantation, arthroseitis, osteomalacia,
osteopenia, osteoporosis, ovarian cancer, Behcet's
disease of bone, peptic ulcer, peripheral vessel
20 disease, prostatic cancer, reflux esophagitis, renal
insufficiency, rheumatoid arthritis, schizophrenia,
sepsis, septic shock, severe systemic fungal infectious
disease, small cell lung cancer, spinal injury, stomach
cancer, systemic lupus erythematosus, transient
25 cerebral ischemia, tuberculosis, cardiac valve failure,
vascular/multiple infarction dementia, wound healing,
insomnia, arthritis, pituitary hormone secretion
disorder, pollakiuria, uremia, neurodegenerative
disease, etc.

30 In addition, the antibody of the present invention
having the effect of neutralizing the polypeptide of
the present invention or the receptor protein of the
present invention can also be used as the
therapeutic/prophylactic agent for macular edema
35 cystoid.

Moreover, since the polypeptide of the present invention or the receptor protein of the present invention are associated with secretion control of somatostatin, the antibody of the present invention
5 having the effect of neutralizing the polypeptide of the present invention or the receptor protein of the present invention are useful as:

(1) therapeutic agents for tumors such as acromegaly, TSH-producing tumor, non-secretory (non-
10 functional) pituitary tumor, ectopic ACTH (adrenocorticotropine)-producing tumor, medullary thyroid cancer, VIP-producing tumor, glucagon-producing tumor, gastrin-producing tumor, insulinoma, carcinoid tumor, etc.;

15 (2) therapeutic agents for insulin-dependent or insulin-independent diabetes mellitus, or various diseases associated with the diabetes, i.e., diabetic complications (e.g., diabetic retinopathy, diabetic nephropathy, diabetic neuropathy, Down's syndrome,
20 orthostatic hypotension, etc.);

(3) agents for improving hyperinsulinism or for the treatment of obesity, bulimia, etc. caused by the suppression of appetite;

25 (4) therapeutic agents for acute pancreatitis, chronic pancreatitis, pancreatic/intestinal fistula, hemorrhagic ulcer, peptic ulcer, gastritis, hyperchylia, reflux esophagitis, etc.;

30 (5) agents for alleviating various conditions accompanied by *Helicobacter pylori* bacterial infections (e.g., an agent for suppressing accentuated gastrin secretion, etc.);

(6) agents for suppressing secretion of amylase accompanied by endoscopic cholangio pancreatography and for the postoperative treatment in pancreas surgery;

(7) agents for the treatment of diarrhea caused by reduced absorption or accentuated secretion in small intestine or abnormal motility of digestive tract (Short bowel syndrome, etc.), diarrhea caused by drugs in chemotherapy of cancer, etc., diarrhea caused by congenital small intestine atrophy, diarrhea caused by neuro-endocrinal tumor such as VIP-producing tumor, etc., diarrhea caused by AIDS, diarrhea caused by graft-versus-host reaction accompanied by spinal transplant, etc., diarrhea caused by diabetes mellitus, diarrhea caused by blocking nervous plexus in the abdominal cavity, diarrhea caused by systemic sclerosis, diarrhea caused by eosinophilia, etc.;

(8) agents for the treatment of Dumping syndrome, hypersensitive colitis, Crohn's disease, inflammatory bowel disease, etc.;

(9) agents for the treatment of tumor or cancer (e.g., thyroid cancer, colon cancer, breast cancer, prostatic cancer, small cell lung cancer, non-small cell lung cancer, pancreatic cancer, gastric cancer, bile duct cancer, liver cancer, bladder cancer, ovary cancer, melanoma, osteosarcoma, chondrosarcoma, malignant pheochromocytoma, neuroblastoma, brain tumor, thymoma, kidney cancer, etc.), leukemia (e.g., leukemia/chronic lymphoid leukemia of basophil leukocyte, chronic myeloid leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, etc.), these agents may also be used alone or in combination with other carcinostatic agents (e.g., tamoxifen, LHRH agonist, LHRH antagonist, interferon- α , β and γ , interleukin-2, etc.);

(10) agents for the prevention and treatment of hypertrophic cardiomyopathy, arteriosclerosis, valvular disease, myocardial infarction (especially myocardial infarction after percutaneous transluminal angioplasty) or regeneration of blood vessels;

(11) agents for the treatment of hemorrhage in esophageal venous cancer, cirrhosis or peripheral vessel disease;

(12) agents for the treatment of disease
5 accompanied by regulation of secretion of physiologically active substances acting on the immune system, such as systemic or regional inflammation (e.g., multiple arthritis, rheumatoid arthritis, psoriasis, sunburn, eczema, allergy (e.g., asthma, atopic
10 dermatitis, allergic rhinitis, etc.), etc.;

(13) agents for the treatment of, for example, dementia (e.g., Alzheimer's disease, Alzheimer's senile dementia, vascular/multiple dementia, etc.), schizophrenia, epilepsy, depression, general anxiety
15 disorder, sleeping disorder, multiple sclerosis, etc.;

(14) agents for the treatment of eye disease (e.g., glaucoma, etc.), etc.;

(15) agents for the prevention and treatment of acute bacterial meningitis, acute viral encephalitis,
20 adult respiratory distress syndrome, bacterial pneumonia, severe systemic fungal infectious disease, tuberculosis, spinal injury, bone fracture, hepatic insufficiency, pneumonia, alcoholic hepatitis, hepatitis A, hepatitis B, hepatitis C, AIDS infectious
25 disease, human papilloma virus infectious disease, influenza infectious disease, cancer metastasis, multiple myeloma, osteomalacia, osteoporosis, Behcet's disease of bone, nephritis, renal insufficiency, sepsis, septic shock, hypercalcemia, hypercholesterolemia,
30 hyperglyceridemia, hyperlipemia, systemic lupus erythematosus, transient cerebral ischemia, alcoholic hepatitis, etc.

(16) Furthermore, the antibody of the present invention is also used for healing organ
35 transplantation, burn, wound, alopecia, etc.

(17) The antibody of the present invention is also useful as analgesics for suppression or alleviation of chronic or acute pains (pains accompanied by, e.g., postoperative pain, inflammatory pain, toothache, bone disease (e.g., arthritis, rheumatoid, osteoporosis, etc.)).

The pharmaceutical agent comprising the antibody of the present invention for the treatment and prevention of the aforesaid diseases may be administered orally or parenterally to human or mammal (e.g., rat, rabbit, sheep, swine, bovine, cat, dog, monkey, etc.) as a liquid preparation in its original form, or as a pharmaceutical composition in an appropriate drug form. The dose varies depending on subject to be administered, target disease, conditions, route for administration, etc.; for example, when used for the treatment and prevention of adult patient with neuropathy, the antibody of the present invention is intravenously administered normally in the dose of about 0.01 mg to about 20 mg/kg body weight, preferably about 1.0 to about 10 mg/kg body weight, and more preferably about 0.1 to about 5 mg per day once to about 5 times a day, preferably once to about 3 times. In parenteral administration in other route and in oral administration, a dose similar to those given above can be administered. Where conditions are serious, the dose may be increased depending on the conditions.

The antibody of the present invention may be administered in itself or as an appropriate pharmaceutical composition. The pharmaceutical composition used for the administration described above contains a pharmacologically acceptable carrier with the aforesaid compounds or salts thereof, a diluent or excipient. Such a composition is provided in the

preparation suitable for oral or parenteral administration.

That is, examples of the composition for oral administration include solid or liquid preparations, specifically, tablets (including dragees and film-coated tablets), pills, granules, powdery preparations, capsules (including soft capsules), syrup, emulsions, suspensions, etc. Such a composition is manufactured by publicly known methods and contains a vehicle, a diluent or an excipient conventionally used in the field of pharmaceutical preparations. Examples of the vehicle or excipient for tablets are lactose, starch, sucrose, magnesium stearate, etc.

Examples of the composition for parenteral administration that can be used are injections, suppositories, etc. and the injections include the form of intravenous, subcutaneous, transcutaneous, intramuscular and drip injections. Such injections are prepared by publicly known methods, e.g., by dissolving, suspending or emulsifying the aforesaid antibody or its salts in a sterile aqueous or oily liquid medium. For the aqueous medium for injection, for example, physiological saline and isotonic solutions containing glucose and other adjuvant, etc. are used. Appropriate dissolution aids, for example, alcohol (e.g. ethanol), polyalcohol (e.g. propylene glycol, polyethylene glycol), nonionic surfactant (e.g. polysorbate 80TM, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)) may be used in combination. For the oily solution, for example, sesame oil, soybean oil and the like are used, and dissolution aids such as benzyl benzoate and benzyl alcohol may be used in combination. The thus-prepared liquid for injection is normally filled in an appropriate ampoule. The suppository used for rectal administration is prepared by mixing the

present invention) can be created by transfecting a desired DNA into an unfertilized egg, a fertilized egg, a spermatozoon, a germinal cell containing a primordial germinal cell thereof, or the like, preferably in the embryogenic stage in the development of a non-human mammal (more preferably in the single cell or fertilized cell stage and generally before the 8-cell phase), by standard means, such as the calcium phosphate method, the electric pulse method, the lipofection method, the agglutination method, the microinjection method, the particle gun method, the DEAE-dextran method etc. In addition, it is possible to transfect the exogenous DNA of the present invention into a somatic cell, a living organ, a tissue cell, or the like by the DNA transfection methods, and utilize the transformant for cell culture, tissue culture, etc. In addition, these cells may be fused with the above-described germinal cell by a publicly known cell fusion method to create the transgenic animal of the present invention.

Examples of the non-human mammal that can be used include bovine, swine, sheep, goat, rabbits, dogs, cats, guinea pigs, hamsters, rats, mice and the like. Above all, preferred are rodents, especially mice (e.g., C57Bl/6 strain, DBA2 strain, etc. for a pure line and for a cross line, B6C3F₁ strain, BDF₁ strain B6D2F₁ strain, BALB/c strain, ICR strain, etc.) or rats (Wistar, SD, etc.), since they are relatively short in ontogeny and life cycle from a standpoint of creating model animals for human disease.

"Mammals" in a recombinant vector that can be expressed in the mammals include the aforesaid non-human mammals and human.

The exogenous DNA of the present invention refers to the DNA of the present invention that is once

isolated and extracted from mammals, not the DNA of the present invention inherently possessed by the non-human mammals.

5 The mutant DNA of the present invention includes mutants resulting from variation (e.g., mutation, etc.) in the base sequence of the original DNA of the present invention, specifically DNAs resulting from base addition, deletion, substitution with other bases, etc. and further including abnormal DNA.

10 The abnormal DNA is intended to mean DNA that expresses the abnormal polypeptide or receptor protein of the present invention and exemplified by DNA that expresses a polypeptide for suppressing the functions of the normal polypeptide or receptor protein of the present invention.

The exogenous DNA of the present invention may be any one of those derived from a mammal of the same species as, or a different species from, the mammal as the target animal. In transfecting the DNA of the present invention, it is generally advantageous to use the DNA as a DNA construct in which the DNA is ligated downstream a promoter capable of expressing the DNA in the target animal. For example, in the case of transfecting the human DNA of the present invention, a DNA transgenic mammal that expresses the DNA of the present invention to a high level, can be prepared by microinjecting a DNA construct (e.g., vector, etc.) ligated with the human DNA of the present invention into a fertilized egg of the target non-human mammal downstream various promoters which are capable of expressing the DNA derived from various mammals (e.g., rabbits, dogs, cats, guinea pigs, hamsters, rats, mice, etc.) bearing the DNA of the present invention highly homologous to the human DNA.

preproencephalin A, vasopressin, etc. Among them, cytomegalovirus promoters, human polypeptide elongation factor 1 α (EF-1 α) promoters, human and chicken β actin promoters etc., which protein can highly express in the whole body are preferred.

It is preferred that the vectors described above have a sequence for terminating the transcription of the desired messenger RNA in the DNA transgenic animal (generally termed terminator); for example, a sequence of each DNA derived from viruses and various mammals. SV40 terminator of the simian virus, etc. are preferably used.

In addition, for the purpose of increasing the expression of the desired exogenous DNA to a higher level, the splicing signal and enhancer region of each DNA, a portion of the intron of an eukaryotic DNA may also be ligated at the 5' upstream of the promoter region, or between the promoter region and the translational region, or at the 3' downstream of the translational region, depending upon purposes.

The translational region for the normal polypeptide or receptor protein of the present invention can be obtained using as a starting material the entire genomic DNA or its portion of liver, kidney, thyroid cell or fibroblast origin from human or various mammals (e.g., rabbits, dogs, cats, guinea pigs, hamsters, rats, mice, etc.) or of various commercially available genomic DNA libraries, or using complementary DNA prepared by a publicly known method from RNA of liver, kidney, thyroid cell or fibroblast origin as a starting material. In addition, an exogenous abnormal DNA can be obtained using complementary DNA prepared by a publicly known method from RNA of human fibroblast origin as a starting material. Alternatively, the translational region for a normal polypeptide

translational region obtained by the cell or tissue described above can be made variant by point mutagenesis.

5 The translational region can be prepared by a conventional DNA engineering technique in which the DNA is ligated downstream the aforesaid promoter and if desired, upstream the translation termination site, as a DNA construct capable of being expressed in the transgenic animal.

10 The exogenous DNA of the present invention is transfected at the fertilized egg cell stage in a manner such that the DNA is certainly present in all the germinal cells and somatic cells of the target mammal. The fact that the exogenous DNA of the present invention is present in the germinal cells of the animal prepared by DNA transfection means that all offspring of the prepared animal will maintain the exogenous DNA of the present invention in all of the germinal cells and somatic cells thereof. The offspring of the animal that inherits the exogenous DNA of the present invention also have the exogenous DNA in all of the germinal cells and somatic cells thereof.

20 The non-human mammal in which the normal exogenous DNA of the present invention has been transfected can be passaged as the DNA-bearing animal under ordinary rearing environment, by confirming that the exogenous DNA is stably retained by mating.

By the transfection of the exogenous DNA of the present invention at the fertilized egg cell stage, the DNA is retained to be excess in all of the germinal and somatic cells. The fact that the exogenous DNA of the present invention is excessively present in the germinal cells of the prepared animal after transfection means that the DNA of the present invention is excessively present in all of the germinal

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cells and somatic cells thereof. The offspring of the animal that inherits the exogenous DNA of the present invention have excessively the DNA of the present invention in all of the germinal cells and somatic cells thereof.

By obtaining a homozygotic animal having the transfected DNA in both of homologous chromosomes and mating a male and female of the animal, all offspring can be passaged to retain the DNA.

In a non-human mammal bearing the normal DNA of the present invention, the normal DNA of the present invention has expressed to a high level, and may eventually develop the function inactive type inadaptability of the polypeptide of the present invention or the receptor protein of the present invention by accelerating the functions of endogenous normal DNA. Therefore, the animal can be utilized as a pathologic model animal for such a disease. Specifically, using the normal DNA transgenic animal of the present invention, it is possible to elucidate the mechanism of the function inactive type inadaptability of the polypeptide or the receptor protein of the present invention and the pathological mechanism of the disease associated with the receptor protein of the present invention and to determine how to treat the disease.

Further, since a mammal transfected the exogenous normal DNA of the present invention exhibits an increasing symptom of the polypeptide or the receptor protein of the present invention librated, the animal is usable for screening of treatment agent for the disease associated with the polypeptide or the receptor protein of the present invention.

On the other hand, non-human mammal having the exogenous abnormal DNA of the present invention can be

5 passed under normal breeding conditions as the DNA-
 bearing animal by confirming the stable retaining of
 the exogenous DNA via crossing. Further, the exogenous
 DNA to be subjected can be utilized as a starting
 10 material by inserting the DNA into the plasmid
 described above. The DNA construct with promoter can
 be prepared with conventional DNA engineering
 techniques. The transfection of the abnormal DNA of
 the present invention at the fertilized egg cell stage
 15 is preserved to be present in all of the germinal and
 somatic cells of the mammals to be subjected. The fact
 that the abnormal DNA of the present invention is
 present in the germinal cells of the animal after DNA
 transfection means that all of the offspring of the
 20 prepared animal have the abnormal DNA of the present
 invention in all of the germinal and somatic cells.
 Such an offspring passed the exogenous DNA of the
 present invention contains the abnormal DNA of the
 present invention in all of the germinal and somatic
 cells. A homozygous animal having the introduced DNA
 on both of homologous chromosomes can be acquired and
 then by mating these male and female animals, all the
 offspring can be bled to have the DNA.

25 Since non-human mammal having the abnormal DNA of
 the present invention may express the abnormal DNA of
 the present invention at a high level, the animal may
 be the function inactivation type inadaptability of the
 polypeptide or the receptor protein of the present
 invention by inhibiting the function of the endogenous
 30 normal DNA and can be utilized as its disease model
 animal. For example, using the abnormal DNA-
 transferred animal of the present invention, it is
 possible to elucidate the mechanism of inadaptability
 of the polypeptide or the receptor protein of the

present invention and to perform to study a method for treatment of this disease.

More specifically, the transgenic animal of the present invention expressing the abnormal DNA of the present invention to a high level is also expected to serve as an experimental model for the elucidation of the mechanism of the functional inhibition (dominant negative effect) of normal polypeptide by the abnormal polypeptide of the present invention in the function inactive type inadaptability of the polypeptide of the present invention or the receptor protein of the present invention.

A mammal bearing the abnormal exogenous DNA of the present invention is also expected to serve for screening a candidate drug for the treatment of the function inactive type inadaptability of the polypeptide of the present invention or the receptor protein of the present invention, since the polypeptide of the present invention or the receptor protein of the present invention is increased in such an animal in its free form.

Other potential applications of two kinds of the transgenic animals described above include:

- (1) use as a cell source for tissue culture;
- (2) elucidation of the relation to a polypeptide that is specifically expressed or activated by the polypeptide of the present invention or the receptor protein of the present invention, by direct analysis of DNA or RNA in tissue of the DNA transgenic animal of the present invention or by analysis of the polypeptide tissue expressed by the DNA;

- (3) research in the function of cells derived from tissues that are cultured usually only with difficulty, using cells of tissue bearing the DNA cultured by a standard tissue culture technique;

an effective research material for the polypeptide of the present invention or the receptor protein of the present invention and for elucidating the function and effect thereof.

5 To develop a therapeutic drug for the treatment of diseases associated with the polypeptide of the present invention or the receptor protein of the present invention, including the function inactive type inadaptability of the polypeptide of the present invention or the receptor protein of the present invention, using the DNA transgenic animal of the present invention, an effective and rapid method for screening can be provided by using the method for inspection and the method for quantification, etc.
10 described above. It is also possible to investigate and develop a method for DNA therapy for the treatment of diseases associated with the polypeptide of the present invention or the receptor protein of the present invention, using the DNA transgenic animal of the present invention or a vector capable of expressing the exogenous DNA of the present invention.
15
20

(8) Knockout animal

The present invention provides a non-human mammal embryonic stem cell bearing the DNA of the present invention inactivated and a non-human mammal deficient in expressing the DNA of the present invention.
25

Thus, the present invention provides:

- (1) a non-human embryonic stem cell in which the DNA of the present invention is inactivated;
30
- (2) an embryonic stem cell according to (1), wherein the DNA is inactivated by introducing a reporter gene (e.g., β -galactosidase gene derived from *Escherichia coli*);

(3) an embryonic stem cell according to (1), which is resistant to neomycin;

(4) an embryonic stem cell according to (1), wherein the non-human mammal is a rodent;

5 (5) an embryonic stem cell according to (4), wherein the rodent is mouse;

(6) a non-human mammal deficient in expressing the DNA of the present invention, wherein the DNA of the present invention is inactivated;

10 (7) a non-human mammal according to (5), wherein the DNA is inactivated by inserting a reporter gene (e.g., β -galactosidase derived from *Escherichia coli*) therein and the reporter gene is capable of being expressed under control of a promoter for the DNA of the present invention;

(8) a non-human mammal according to (6), which is a rodent;

(9) a non-human mammal according to (8), wherein the rodent is mouse; and,

20 (10) a method for screening a compound that accelerates or inhibits the promoter activity for the DNA of the present invention, which comprises administering a test compound to the mammal of (7) and detecting expression of the reporter gene.

25 The non-human mammal embryonic stem cell in which the DNA of the present invention is inactivated refers to a non-human mammal embryonic stem cell that suppresses the ability of the non-human mammal to express the DNA by artificially mutating the DNA of the present invention, or the DNA has no substantial
30 ability to express the polypeptide of the present invention or the receptor protein of the present invention (hereinafter sometimes referred to as the knockout DNA of the present invention) by substantially
35 inactivating the activities of the polypeptide of the

present invention or the receptor protein of the present invention encoded by the DNA (hereinafter merely referred to as ES cell).

As the non-human mammal, the same examples as described above apply.

Techniques for artificially mutating the DNA of the present invention include deletion of a part or all of the DNA sequence and insertion of or substitution with other DNA, by genetic engineering. By these variations, the knockout DNA of the present invention may be prepared, for example, by shifting the reading frame of a codon or by disrupting the function of a promoter or exon.

Specifically, the non-human mammal embryonic stem cell in which the DNA of the present invention is inactivated (hereinafter merely referred to as the ES cell with the DNA of the present invention inactivated or the knockout ES cell of the present invention) can be obtained by, for example, isolating the DNA of the present invention that the desired non-human mammal possesses, inserting a DNA fragment having a DNA sequence constructed by inserting a drug resistant gene such as a neomycin resistant gene or a hygromycin resistant gene, or a reporter gene such as lacZ (β -galactosidase gene) or cat (chloramphenicol acetyltransferase gene), etc. into its exon site thereby to disable the functions of exon, or integrating to a chromosome of the subject animal by, e.g., homologous recombination, a DNA sequence which terminates gene transcription (e.g., polyA additional signal, etc.) in the intron between exons to, thus inhibit the synthesis of complete messenger RNA and eventually destroy the gene (hereinafter simply referred to as targeting vector). The thus-obtained ES cells to Southern hybridization analysis with a DNA

sequence on or near the DNA of the present invention as a probe, or to PCR analysis with a DNA sequence on the targeting vector and another DNA sequence near the DNA of the present invention which is not included in the targeting vector as primers, to select the knockout ES cell of the present invention.

The parent ES cells to inactivate the DNA of the present invention by homologous recombination, etc. may be of a strain already established as described above, or may be originally established in accordance with a modification of the known method by Evans and Kaufman *supra*. For example, in the case of mouse ES cells, currently it is common practice to use ES cells of the 129 strain. However, since their immunological background is obscure, the C57BL/6 mouse or the BDF1 mouse (F1 hybrid between C57BL/6 and DBA/2), wherein the low ovum availability per C57BL/6 in the C57BL/6 mouse has been improved by crossing with DBA/2, may be preferably used, instead of obtaining a pure line of ES cells with the clear immunological genetic background and for other purposes. The BDF1 mouse is advantageous in that, when a pathologic model mouse is generated using ES cells obtained therefrom, the genetic background can be changed to that of the C57BL/6 mouse by back-crossing with the C57BL/6 mouse, since its background is of the C57BL/6 mouse, as well as being advantageous in that ovum availability per animal is high and ova are robust.

In establishing ES cells, blastocytes at 3.5 days after fertilization are commonly used. In the present invention, embryos are preferably collected at the 8-cell stage, after culturing until the blastocyte stage, the embryos are used to efficiently obtain a large number of early stage embryos.

Although the ES cells used may be of either sex, male ES cells are generally more convenient for generation of a germ cell line chimera and are therefore preferred. It is also desirable that sexes be
5 identified as soon as possible to save painstaking culture time.

Methods for sex identification of the ES cell include the method in which a gene in the sex-determining region on the Y-chromosome is amplified by
10 the PCR process and detected. When this method is used, one colony of ES cells (about 50 cells) is sufficient for sex-determination analysis, which karyotype analysis, for example G-banding method, requires about 10^6 cells; therefore, the first selection of ES cells
15 at the early stage of culture can be based on sex identification, and male cells can be selected early, which saves a significant amount of time at the early stage of culture.

Second selection can be achieved by, for example,
20 number of chromosome confirmation by the G-banding method. It is usually desirable that the chromosome number of the obtained ES cells be 100% of the normal number. However, when it is difficult to obtain the cells having the normal number of chromosomes due to
25 physical operation etc. in cell establishment, it is desirable that the ES cell be again cloned to a normal cell (e.g., in mouse cells having the number of chromosomes being $2n = 40$) after the gene of the ES cells is rendered knockout.

30 Although the embryonic stem cell line thus obtained shows a very high growth potential, it must be subcultured with great care, since it tends to lose its ontogenic capability. For example, the embryonic stem cell line is cultured at about 37°C in a carbon dioxide
35 incubator (preferably about 5% carbon dioxide and about

95% air, or about 5% oxygen, about 5% carbon dioxide and 90% air) in the presence of LIF (1-10000 U/ml) on appropriate feeder cells such as STO fibroblasts, treated with a trypsin/EDTA solution (normally about
5 0.001 to about 0.5% trypsin/about 0.1 to about 5 mM EDTA, preferably about 0.1% trypsin/1 mM EDTA) at the time of passage to obtain separate single cells, which are then seeded on freshly prepared feeder cells. This passage is normally conducted every 1 to 3 days; it is
10 desirable that cells be observed at passage and cells found to be morphologically abnormal in culture, if any, be abandoned.

Where ES cells are allowed to reach a high density in mono-layers or to form cell aggregates in suspension
15 under appropriate conditions, they will spontaneously differentiate to various cell types, for example, pariental and visceral muscles, cardiac muscle or the like (M. J. Evans and M. H. Kaufman, Nature, 292, 154, 1981; G. R. Martin, Proc. Natl. Acad. Sci. U.S.A., 78,
20 7634, 1981; T. C. Doetschman et al., Journal of Embryology Experimental Morphology, 87, 27, 1985). The cells deficient in expression of the DNA of the present invention, which are obtainable from the differentiated
25 ES cells of the present invention are useful for studying the functions of the polypeptide of the present invention or the receptor protein of the present invention cytologically or molecular biologically.

The non-human mammal deficient in expression of
30 the DNA of the present invention can be identified from a normal animal by measuring the mRNA amount in the subject animal by a publicly known method, and indirectly comparing the degrees of expression.

As the non-human mammal, the same examples supra
35 apply.

obtained by crossing between such a chimeric animal and
 a normal animal, e.g., by coat color identification,
 etc. The individuals thus obtained are normally
 deficient in heterozygous expression of the peptide of
 5 the present invention. The individuals deficient in
 homozygous expression of the polypeptide of the present
 invention or the receptor protein of the present
 invention can be obtained from offspring of the
 intercross between the heterozygotes.

10 When an oocyte or egg cell is used, a DNA solution
 may be injected, e.g., to the pronucleus by
 microinjection thereby to obtain a transgenic non-human
 mammal having a targeting vector introduced in a
 chromosome thereof. From such transgenic non-human
 15 mammals, those having a mutation at the locus of the
 DNA of the present invention can be obtained by
 selection based on homologous recombination.

As described above, individuals in which the DNA
 of the present invention is rendered knockout permit
 20 passage rearing under ordinary rearing conditions,
 after the individuals obtained by their crossing have
 proven to have been knockout.

Furthermore, the genital system may be obtained
 and maintained by conventional methods. That is, by
 25 crossing male and female animals each having the
 inactivated DNA, homozygote animals having the
 inactivated DNA in both loci can be obtained. The
 homozygotes thus obtained may be reared so that one
 normal animal and two or more homozygotes are produced
 30 from a mother animal to efficiently obtain such
 homozygotes. By crossing male and female heterozygotes,
 homozygotes and heterozygotes having the inactivated
 DNA are proliferated and passaged.

The non-human mammal embryonic stem cell in which
 35 the DNA of the present invention is inactivated is very

useful for preparing a non-human mammal deficient in expression of the DNA of the present invention.

Since the non-human mammal in which the DNA of the present invention is inactivated lacks various biological activities derived from the polypeptide of the present invention or the receptor protein of the present invention, such an animal can be a disease model suspected of inactivated biological activities of the polypeptide of the present invention or the receptor protein of the present invention and thus, offers an effective study to investigate causes for and therapy for these diseases.

(8a) Method for screening of compounds having therapeutic/prophylactic effects for diseases caused by deficiency, damages, etc. of the DNA of the present invention

The non-human mammal deficient in expression of the DNA of the present invention can be employed for screening of compounds having therapeutic/prophylactic effects for diseases caused by deficiency, damages, etc. of the DNA of the present invention.

That is, the present invention provides a method for screening of a compound having therapeutic/prophylactic effects for diseases caused by deficiency, damages, etc. of the DNA of the present invention, which comprises administering a test compound to the non-human mammal deficient in expression of the DNA of the present invention and observing and measuring a change occurred in the animal.

As the non-human mammal deficient in expression of the DNA of the present invention which can be employed for the screening method, the same examples as given hereinabove apply.

prophylactic effect for macular edema cystoid; and
 furthermore, a compound useful as a therapeutic and
 prophylactic effect as:

- (1) therapeutic agents for tumors such as
 5 acromegaly, TSH-producing tumor, non-secretory (non-
 functional) pituitary tumor, ectopic ACTH
 (adrenocorticotropine)-producing tumor, medullary
 thyroid cancer, VIP-producing tumor, glucagon-producing
 tumor, gastrin-producing tumor, insulinoma, carcinoid
 10 tumor, etc.;
- (2) therapeutic agents for insulin-dependent or
 insulin-independent diabetes mellitus, or various
 diseases associated with the diabetes, i.e., diabetic
 complications (e.g., diabetic retinopathy, diabetic
 15 nephropathy, diabetic neuropathy, Down's syndrome,
 orthostatic hypotension, etc.);
- (3) agents for improving hyperinsulinism or for
 the treatment of obesity, bulimia, etc. caused by the
 suppression of appetite;
- 20 (4) therapeutic agents for acute pancreatitis,
 chronic pancreatitis, pancreatic/intestinal fistula,
 hemorrhagic ulcer, peptic ulcer, gastritis, hyperchylia,
 reflux esophagitis, etc.;
- (5) agents for alleviating various conditions
 25 accompanied by Helicobacter pylori bacterial infections
 (e.g., an agent for suppressing accentuated gastrin
 secretion, etc.);
- (6) agents for suppressing secretion of amylase
 accompanied by endoscopic cholangio pancreatography and
 30 for the postoperative treatment in pancreas surgery;
- (7) agents for the treatment of diarrhea caused by
 reduced absorption or accentuated secretion in small
 intestine or abnormal motility of digestive tract
 (Short bowel syndrome, etc.), diarrhea caused by drugs
 35 in chemotherapy of cancer, etc., diarrhea caused by

congenital small intestine atrophy, diarrhea caused by neuro-endocrinal tumor such as VIP-producing tumor, etc., diarrhea caused by AIDS, diarrhea caused by graft-versus-host reaction accompanied by spinal
 5 transplant, etc., diarrhea caused by diabetes mellitus, diarrhea caused by blocking nervous plexus in the abdominal cavity, diarrhea caused by systemic sclerosis, diarrhea caused by eosinophilia, etc.;

(8) agents for the treatment of Dumping syndrome,
 10 hypersensitive colitis, Crohn's disease, inflammatory bowel disease, etc.;

(9) agents for the treatment of tumor or cancer (e.g., thyroid cancer, colon cancer, breast cancer, prostatic cancer, small cell lung cancer, non-small
 15 cell lung cancer, pancreatic cancer, gastric cancer, bile duct cancer, liver cancer, bladder cancer, ovary cancer, melanoma, osteosarcoma, chondrosarcoma, malignant pheochromocytoma, neuroblastoma, brain tumor, thymoma, kidney cancer, etc.), leukemia (e.g.,
 20 leukemia/chronic lymphoid leukemia of basophil leukocyte, chronic myeloid leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, etc.), these agents may also be used alone or in combination with other carcinostatic agents (e.g., tamoxifen, LHRH agonist, LHRH antagonist,
 25 interferon- α , β and γ , interleukin-2, etc.);

(10) agents for the prevention and treatment of hypertrophic cardiomyopathy, arteriosclerosis, valvular disease, myocardial infarction (especially myocardial infarction after percutaneous transluminal angioplasty)
 30 or regeneration of blood vessels;

(11) agents for the treatment of hemorrhage in esophageal venous cancer, cirrhosis or peripheral vessel disease;

(12) agents for the treatment of disease
 35 accompanied by regulation of secretion of

In the screening method *supra*, when a test compound is administered to an animal to be tested and found to reduce the blood sugar level of the animal to at least about 10%, preferably at least about 30% and more preferably at least about 50%, the test compound can be selected to be a compound having a therapeutic and prophylactic effect for the diseases *supra*.

The compound obtained using the above screening method is a compound selected from the test compounds described above and exhibits a therapeutic and prophylactic effect for the diseases caused by deficiencies, damages, etc. of the polypeptide of the present invention or the receptor protein of the present invention. Therefore, the compound can be employed as a safe and low toxic drug for the treatment and prevention of these diseases. Furthermore, compounds derived from such a compound obtained by the screening *supra* can be likewise employed.

The compound obtained by the screening above may be used in the form of salts with physiologically acceptable acids (e.g., inorganic acids or organic acids) or bases (e.g., alkali metal salts), preferably in the form of physiologically acceptable acid addition salts. Examples of such salts are salts with inorganic acids (e.g., hydrochloric acid, phosphoric acid, hydrobromic acid, sulfuric acid), salts with organic acids (e.g., acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid, benzenesulfonic acid) and the like.

A pharmaceutical composition comprising the compound obtained by the above screening method or salts thereof may be manufactured in a manner similar to the method for preparing the composition comprising

the polypeptide of the present invention described hereinabove.

Since the pharmaceutical composition thus obtained is safe and low toxic, it can be administered to human
 5 and another mammal (e.g., rat, mouse, guinea pig, rabbit, sheep, swine, bovine, horse, cat, dog, monkey, etc.).

Although the amount of the compound or its salt to be administered varies depending upon particular
 10 disease, subject to be administered, route of administration, etc. in general, for oral administration to an adult (as 60 kg body weight), the compound is administered in an amount of about 0.1 mg/day to about 100 mg/day, preferably about 1.0 mg/day
 15 to about 50 mg/day, more preferably about 1.0 mg to about 20 mg. For parenteral administration to an adult (as 60 kg body weight), it is advantageous to administer the composition in the form of an injectable preparation in an amount of about 0.01 mg/day to about
 20 30 mg/day, preferably about 0.1 mg/day to about 20 mg/day, more preferably about 0.1 mg/day to about 10 mg/day, though the single dosage varies depending upon particular subject, particular disease, etc. As for other animals, the composition can be administered in
 25 the above amount with converting it into that for the body weight of 60 kg.

(8b) Method for screening a compound that accelerates or inhibits the activities of a promoter to the DNA of the present invention

30 The present invention provides a method for screening a compound that accelerates or inhibits the activities of a promoter to the DNA of the present invention or salts thereof, which comprises administering a test compound to a non-human mammal

deficient in expression of the DNA of the present invention and detecting expression of the reporter gene.

In the screening method *supra*, the non-human mammal deficient in expression of the DNA of the present invention is selected from the aforesaid non-human mammal deficient in expression of the DNA of the present invention, as an animal in which the DNA of the present invention is inactivated by introducing a reporter gene and the reporter gene is expressed under control of a promoter to the DNA of the present invention.

The same examples of the test compound apply to specific compounds used for the screening.

As the reporter gene, the same specific examples apply to this screening method. Preferably employed are β -galactosidase (lacZ), soluble alkaline phosphatase gene, luciferase gene and the like.

Since a reporter gene is present under control of a promoter to the DNA of the present invention in the non-human mammal deficient in expression of the DNA of the present invention wherein the DNA of the present invention is substituted with the reporter gene, the activity of the promoter can be detected by tracing expression of a substance encoded by the reporter gene.

When a part of the DNA region encoding the polypeptide of the present invention or the receptor protein of the present invention is substituted with, e.g., β -galactosidase gene (lacZ) derived from *Escherichia coli*, β -galactosidase is expressed in a tissue where the polypeptide of the present invention or the receptor protein of the present invention should originally be expressed, instead of the polypeptide or receptor protein of the present invention. Thus, the state of expression of the polypeptide or the receptor protein of the present invention can be readily

observed in vivo of an animal by staining with a
 reagent, e.g., 5-bromo-4-chloro-3-indolyl- β -
 galactopyranoside (X-gal) which is substrate for β -
 galactosidase. Specifically, a mouse deficient in the
 5 polypeptide of the present invention or the receptor
 protein of the present invention, or its tissue section
 is fixed with glutaraldehyde, etc. After washing with
 phosphate buffered saline (PBS), the system is reacted
 with a staining solution containing X-gal at room
 10 temperature or about 37°C for approximately 30 minutes
 to an hour. After the β -galactosidase reaction is
 terminated by washing the tissue preparation with 1 mM
 EDTA/PBS solution, the color formed is observed.
 Alternatively, mRNA encoding lacZ may be detected in a
 15 conventional manner.

The compound or salts thereof obtained using the
 screening method *supra* are compounds that are selected
 from the test compounds described above and that
 accelerate or inhibit the promoter activity to the DNA
 20 of the present invention.

The compound obtained by the screening method
 above may be used in the form of salts with
 physiologically acceptable acids (e.g., inorganic acids
 or organic acids) or bases (e.g., alkali metal salts),
 25 preferably in the form of physiologically acceptable
 acid addition salts. Examples of such salts are salts
 with inorganic acids (e.g., hydrochloric acid,
 phosphoric acid, hydrobromic acid, sulfuric acid),
 salts with organic acids (e.g., acetic acid, formic
 30 acid, propionic acid, fumaric acid, maleic acid,
 succinic acid, tartaric acid, citric acid, malic acid,
 oxalic acid, benzoic acid, methanesulfonic acid,
 benzenesulfonic acid) and the like.

Since the compounds or salts thereof that
 35 accelerate or inhibit the promoter activity to the DNA

of the present invention can accelerate or inhibit the
 expression of the polypeptide of the present invention
 or the receptor protein of the present invention or can
 accelerate or inhibit the functions of the polypeptide
 5 of the present invention or the receptor protein of the
 present invention, they are useful as safe and low
 toxic drugs for the treatment/prevention of diseases
 such as hypertension, autoimmune disease, heart failure,
 cataract, glaucoma, acute bacterial meningitis, acute
 10 myocardial infarction, acute pancreatitis, acute viral
 encephalitis, adult respiratory distress syndrome,
 alcoholic hepatitis, Alzheimer's disease, asthma,
 arteriosclerosis, atopic dermatitis, bacterial
 pneumonia, bladder cancer, fracture, breast cancer,
 15 bulimia, polyphagia, burn healing, uterine cervical
 cancer, chronic lymphocytic leukemia, chronic
 myelogenous leukemia, chronic pancreatitis, liver
 cirrhosis, cancer of the colon and rectum (colon cancer,
 rectal cancer), Crohn's disease, dementia, diabetic
 20 complications, diabetic nephropathy, diabetic
 neuropathy, diabetic retinopathy, gastritis,
 Helicobacter pylori bacterial infectious disease,
 hepatic insufficiency, hepatitis A, hepatitis B,
 hepatitis C, hepatitis, herpes simplex virus infectious
 25 disease, varicellazoster virus infectious disease,
 Hodgkin's disease, AIDS infectious disease, human
 papilloma virus infectious disease, hypercalcemia,
 hypercholesterolemia, hyperglyceridemia, hyperlipemia,
 infectious disease, influenza infectious disease,
 30 insulin dependent diabetes mellitus (type I), invasive
 staphylococcal infectious disease, malignant melanoma,
 cancer metastasis, multiple myeloma, allergic rhinitis,
 nephritis, non-Hodgkin's lymphoma, insulin-independent
 diabetes mellitus (type II), non-small cell lung cancer,
 35 organ transplantation, arthroseitis, osteomalacia,

osteopenia, osteoporosis, ovarian cancer, Behcet' s
disease of bone, peptic ulcer, peripheral vessel
disease, prostatic cancer, reflux esophagitis, renal
insufficiency, rheumatoid arthritis, schizophrenia,
5 sepsis, septic shock, severe systemic fungal infectious
disease, small cell lung cancer, spinal injury, stomach
cancer, systemic lupus erythematosus, transient
cerebral ischemia, tuberculosis, cardiac valve failure,
vascular/multiple infarction dementia, wound healing,
10 insomnia, arthritis, pituitary hormone secretion
disorder, pollakiuria, uremia, neurodegenerative
disease, etc.

In addition, the compounds or salts thereof that
accelerate or inhibit the promoter activity to the DNA
15 of the present invention can also be used as a safe and
low toxic therapeutic/prophylactic agent for macular
edema cystoid.

Moreover, the compounds or salts thereof that
accelerate or inhibit the promoter activity to the DNA
20 of the present invention are useful as safe and low
toxic therapeutic/prophylactic agents for diseases,
which are specifically given below:

(1) therapeutic agents for tumors such as
acromegaly, TSH-producing tumor, non-secretory (non-
25 functional) pituitary tumor, ectopic ACTH
(adrenocorticotropine)-producing tumor, medullary
thyroid cancer, VIP-producing tumor, glucagon-producing
tumor, gastrin-producing tumor, insulinoma, carcinoid
tumor, etc.;

(2) therapeutic agents for insulin-dependent or
30 insulin-independent diabetes mellitus, or various
diseases associated with the diabetes, i.e., diabetic
complications (e.g., diabetic retinopathy, diabetic
nephropathy, diabetic neuropathy, Down's syndrome,
35 orthostatic hypotension, etc.);

(3) agents for improving hyperinsulinism or for the treatment of obesity, bulimia, etc. caused by the suppression of appetite;

5 (4) therapeutic agents for acute pancreatitis, chronic pancreatitis, pancreatic/intestinal fistula, hemorrhagic ulcer, peptic ulcer, gastritis, hyperchylia, reflux esophagitis, etc.;

10 (5) agents for alleviating various conditions accompanied by Helicobacter pylori bacterial infections (e.g., an agent for suppressing accentuated gastrin secretion, etc.);

(6) agents for suppressing secretion of amylase accompanied by endoscopic cholangio pancreatography and for the postoperative treatment in pancreas surgery;

15 (7) agents for the treatment of diarrhea caused by reduced absorption or accentuated secretion in small intestine or abnormal motility of digestive tract (Short bowel syndrome, etc.), diarrhea caused by drugs in chemotherapy of cancer, etc., diarrhea caused by
20 congenital small intestine atrophy, diarrhea caused by neuro-endocrinal tumor such as VIP-producing tumor, etc., diarrhea caused by AIDS, diarrhea caused by graft-versus-host reaction accompanied by spinal transplant, etc., diarrhea caused by diabetes mellitus,
25 diarrhea caused by blocking nervous plexus in the abdominal cavity, diarrhea caused by systemic sclerosis, diarrhea caused by eosinophilia, etc.;

(8) agents for the treatment of Dumping syndrome, hypersensitive colitis, Crohn's disease, inflammatory
30 bowel disease, etc.;

(9) agents for the treatment of tumor or cancer (e.g., thyroid cancer, colon cancer, breast cancer, prostatic cancer, small cell lung cancer, non-small cell lung cancer, pancreatic cancer, gastric cancer,
35 bile duct cancer, liver cancer, bladder cancer, ovary

tuberculosis, spinal injury, bone fracture, hepatic insufficiency, pneumonia, alcoholic hepatitis, hepatitis A, hepatitis B, hepatitis C, AIDS infectious disease, human papilloma virus infectious disease, influenza infectious disease, cancer metastasis, multiple myeloma, osteomalacia, osteoporosis, Behcet's disease of bone, nephritis, renal insufficiency, sepsis, septic shock, hypercalcemia, hypercholesterolemia, hyperglyceridemia, hyperlipemia, systemic lupus erythematosus, transient cerebral ischemia, alcoholic hepatitis, etc.;

(16) further use in healing organ transplantation, burn, wound, alopecia, etc.;

(17) analgesics for suppression or alleviation of chronic or acute pains (pains accompanied by, e.g., postoperative pain, inflammatory pain, toothache, bone disease (e.g., arthritis, rheumatoid, osteoporosis, etc.)). In addition, compound derived from the compounds obtained by the screening above may be likewise employed.

A pharmaceutical composition comprising the compounds or salts thereof obtained by the screening method *supra* may be manufactured in a manner similar to the method for preparing the composition comprising the polypeptide of the present invention described hereinabove.

Since the pharmaceutical composition thus obtained is safe and low toxic, it can be administered to human or another mammal (e.g., rat, mouse, guinea pig, rabbit, sheep, swine, bovine, horse, cat, dog, monkey, etc.).

The dose of the compound or salts thereof varies depending on target disease, subject to be administered, method for administration, etc.; for example, in oral administration of the compound that accelerates the promoter activity to the DNA of the present invention,

the dose is normally about 0.1 to about 100 mg,
preferably about 1.0 to about 50 mg, more preferably
about 1.0 to about 20 mg per day for adult (as 60 kg
body weight). In parenteral administration, the single
5 dose varies depending on subject to be administered,
target disease, etc. but it is advantageous to
administer, for example, the compound that accelerates
the functions of the polypeptide of the present
invention intravenously at a daily dose of about 0.01
10 to about 30 mg, preferably about 0.1 to about 20 mg,
more preferably about 0.1 to about 10 mg for adult (as
60 kg body weight). For other animal species, the
corresponding dose as converted per 60 kg weight can be
administered.

15 Turning to the compound that inhibits the promoter
activity to the DNA of the present invention when it is
orally administered, the dose is normally about 0.1 to
about 100 mg, preferably about 1.0 to about 50 mg, more
preferably about 1.0 to about 20 mg per day for adult
20 (as 60 kg body weight). In parenteral administration,
the single dose varies depending on subject to be
administered, target disease, etc. When the compound
that inhibits the promoter activity to the DNA of the
present invention is administered to an adult (as 60 kg
25 body weight) generally in the form of injection, it is
advantageous to administer the compound intravenously
at a daily dose of about 0.01 to about 30 mg,
preferably about 0.1 to about 20 mg, more preferably
about 0.1 to about 10 mg. For other animal species, the
30 corresponding dose as converted per 60 kg weight can be
administered.

As stated above, the non-human mammal deficient in
expression of the DNA of the present invention is
extremely useful for screening the compound or its salt
35 that accelerates or inhibits the activity of a promoter

to the DNA of the present invention and can greatly contribute to the elucidation of causes for various diseases suspected of deficiency in expression of the DNA of the present invention and for the development of prophylactic/therapeutic agent for these diseases.

Furthermore, a so-called transgenic animal (gene transferred animal) can be prepared by using DNA containing a promoter region of the polypeptide of the present invention or the receptor protein of the present invention, ligating genes encoding various proteins downstream and injecting the same into oocyte of an animal. It is then possible to synthesize the polypeptide or protein therein specifically and study its activity in vivo. When an appropriate reporter gene is ligated to the promoter site above and a cell line that express the gene is established, the resulting system can be utilized for exploring a low molecular compound having the action of specifically promoting or inhibiting the in vivo productivity of the polypeptide of the present invention or the receptor protein of the present invention, per se.

(9) Identification of receptor to the polypeptide of the present invention

The receptor to the polypeptide of the present invention can be identified as follows. Most receptors for the physiologically active peptides are of seven-transmembrane type and presently many orphan receptors, which ligands are unknown, are reported. Thus, specific receptors can be identified by expressing these orphan receptors in appropriate cells such as CHO cells, HEK293 cells, etc. and adding the polypeptide of the present invention to the expressed receptors to examine if such a cell stimulating activity as inducing a specific signal transduction is exhibited. Furthermore,

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a gene encoding the receptor can be isolated by inserting genome or cDNA library into appropriate animal cells and adding thereto a radioisotope-labeled polypeptide of the present invention to examine its binding.

Since a gene encoding the physiologically active peptide is characterized often by repeating a sequence motif of the peptide, the present invention further provides a method for identification of an unknown physiologically active peptide or its amides or esters, or salts thereof, by utilizing the characteristic property and also provides a physiologically active peptide obtained by the method, its amides or esters, or salts thereof.

Specific examples of the sequence motif possessed by the physiologically active peptide are RFG (R/K) sequence or RSG (R/K) sequence or RLG (R/K) sequence which is characteristic of the polypeptide of the present invention bearing an RF amide, RS amide or RL amide structure, and a base sequence encoding the amino acid sequence. The DNA sequence capable of encoding such a short amino acid sequence appears accidentally with a considerably high frequency also in those other than the DNA sequence of the physiologically active peptide. By exploring a sequence characterized by repeating such a sequence, DNA encoding a physiologically active peptide can be discovered in a high probability.

More specifically, the desired gene can be obtained by retrieval of database using as a probe the RFG(R/K) sequence or RSG(R/K) sequence or RLG(K/R) sequence or a sequence containing the amino acid sequence and a sequence containing the base sequence encoding the same. Examples of the probe include:

ligand to orphan receptor can be determined by using an anti-RF amide antibody, anti-RS amide antibody or anti-RL amide antibody, wherein a condensed or fractionated animal tissue extract is added to cells capable of expressing an orphan receptor with the ligand being not determined. Since many peptides that contain a common structure other than those having the RF amide, RS amide or RL amide structure are present, this method is applicable also to peptides other than those having the RF amide, RS amide or RL amide structure.

(10) Determination of a ligand (agonist) to the receptor protein of the present invention

The receptor protein of the present invention is useful as a reagent for searching and determining a ligand (agonist) to the receptor protein of the present invention and salts thereof.

That is, the present invention provides a method for determining a ligand to the receptor protein of the present invention, which comprises bringing the receptor protein of the present invention in contact with a test compound.

Examples of compounds to be tested include publicly known ligands (e.g., angiotensin, bombesin, canavanoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purines, vasopressin, oxytocin, PACAP, secretin, glucagon, calcitonin, adrenomedulin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, VIP (vasoactive intestinal and related polypeptide), somatostatin, dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene-related peptide), leukotrienes, pancreastatin, prostaglandins, thromboxane, adenosine, adrenaline, α and β -chemokines (e.g., IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1 α , MIP-1 β , RANTES, etc.), endothelin,

enterogastrin, histamin, neurotensin, TRH, pancreatic polypeptide, galanin, etc.) as well as other substances, for example, tissue extracts and cell culture supernatants from human and mammals (e.g., mice, rats, swine, bovine, sheep, monkeys, etc.). For example, the tissue extract or cell culture supernatant is added to the receptor protein of the present invention and fractionated while assaying the cell stimulating activities to finally give a single ligand.

10 In more detail, the method for determining a ligand of the present invention comprises determining compounds (e.g., peptides, proteins, non-peptide compounds, synthetic compounds, fermentation products, etc.) or salts thereof that bind to the receptor protein of the present invention to provide cell stimulating activities (e.g., the activities that accelerate or suppress arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP production, intracellular cGMP production, inositol phosphate production, change in cell membrane potential, phosphorylation of intracellular proteins, activation of c-fos, pH reduction, etc.), using the receptor of the present invention, or using the constructed recombinant receptor protein expression system in the receptor binding assay.

The method for determining a ligand of the present invention is characterized, for example, by measurement of the amount of the test compound bound to the receptor protein of the present invention or the cell-stimulating activities, etc., when the test compound is brought in contact with the receptor protein of the present invention.

More specifically, the present invention provides the following:

(1) A method for determining a ligand to the receptor protein of the present invention which comprises bringing a labeled test compound in contact with the receptor protein of the present invention and
5 measuring the amount of the labeled test compound bound to the receptor protein;

(2) A method for determining a ligand to the receptor protein of the present invention which comprises bringing a labeled test compound in contact
10 with cells containing the receptor protein of the present invention or with a membrane fraction of the cells and measuring the amount of the labeled test compound bound to the cells or the membrane fraction;

(3) A method for determining a ligand to the receptor protein of the present invention which comprises culturing a transformant containing the DNA encoding the receptor protein of the present invention, bringing a labeled test compound in contact with the receptor protein expressed on the cell membrane by said
15 culturing, and measuring the amount of the labeled test compound bound to the expressed receptor protein;

(4) A method for determining a ligand to the receptor protein of the present invention which comprises bringing a test compound in contact with
25 cells containing the receptor protein of the present invention and measuring the receptor protein-mediated cell stimulating activities (e.g., the activities that promote or suppress arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP production, intracellular cGMP
30 production, inositol phosphate production, change in cell membrane potential, phosphorylation of intracellular proteins, activation of c-fos, pH reduction, etc.); and,

preferred to insert the DNA fragment downstream the polyhedrin promoter of nuclear polyhedrosis virus (NPV), which is a baculovirus having insect hosts, an SV40-derived promoter, a retrovirus promoter, a
5 metallothionein promoter, a human heat shock promoter, a cytomegalovirus promoter, an SR α promoter or the like. The amount and quality of the receptor expressed can be determined by a publicly known method. For example, this determination can be made by the method
10 described in the literature (Nambi, P. et al., J. Biol. Chem., Vol. 267, pp. 19555-19559 (1992)).

Accordingly, the subject containing the receptor protein in the method for determining the ligand may be the receptor protein purified by publicly known method,
15 cells containing the receptor protein or membrane fraction of such cells.

Where cells containing the receptor protein of the present invention are used in the method of the present invention for determination of ligands, the cells may
20 be fixed using glutaraldehyde, formalin etc. The fixation can be made by a publicly known method.

The cells containing the receptor protein of the present invention are host cells that have expressed the receptor protein of the present invention, which
25 host cells include *Escherichia coli*, *Bacillus subtilis*, yeast, insect cells, animal cells and the like.

The cell membrane fraction is a fraction abundant in cell membrane obtained by cell disruption and subsequent fractionation by a publicly known method.
30 Useful cell disruption methods include cell squashing using a Potter-Elvehjem homogenizer, disruption using a Waring blender or Polytron (manufactured by Kinematica Inc.), disruption by ultrasonication, and disruption by cell spraying through thin nozzles under an increased
35 pressure using a French press or the like. Cell

membrane fractionation is effected mainly by fractionation using a centrifugal force, such as centrifugation for fractionation and density gradient centrifugation. For example, cell disruption fluid is centrifuged at a low speed (500 rpm to 3,000 rpm) for a short period of time (normally about 1 to about 10 minutes), the resulting supernatant is then centrifuged at a higher speed (15,000 rpm to 30,000 rpm) normally for 30 minutes to 2 hours. The precipitate thus obtained is used as the membrane fraction. The membrane fraction is rich in the receptor protein expressed and membrane components such as cell-derived phospholipids and membrane proteins.

The amount of the receptor protein in the cells containing the receptor protein and in the membrane fraction is preferably 10^3 to 10^8 molecules per cell, more preferably 10^5 to 10^7 molecules per cell. As the amount of expression increases, the ligand binding activity per unit of membrane fraction (specific activity) increases so that not only the highly sensitive screening system can be constructed but also large quantities of samples can be assayed with the same lot.

To perform the methods (1) through (3) for determination of a ligand to the receptor protein of the present invention, an appropriate receptor fraction and a labeled test compound are required.

The receptor protein fraction is preferably a fraction of naturally occurring receptor protein or a recombinant receptor fraction having an activity equivalent to that of the natural protein. Herein, the term "equivalent activity" is intended to mean a ligand binding activity, a signal transduction activity or the like that is equivalent to that possessed by naturally occurring receptor proteins.

Preferred examples of labeled test compounds include angiotensin, bombesin, canavaninoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purines, vasopressin, oxytocin, PACAP, secretin, glucagon, calcitonin, adrenomedulin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, VIP (vasoactive intestinal polypeptide), somatostatin, dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene-related peptide), leukotrienes, pancreastatin, prostaglandins, thromboxane, adenosine, adrenaline, α and β -chemokines (e.g., IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1 α , MIP-1 β , RANTES, etc.), endothelin, enterogastrin, histamin, neurotensin, TRH, pancreatic polypeptide, galanin, etc.), which are labeled with [^3H], [^{125}I], [^{14}C], [^{35}S], etc.

More specifically, the ligand to the receptor protein of the present invention is determined by the following procedures. First, a standard receptor preparation is prepared by suspending cells containing the receptor protein of the present invention or the membrane fraction thereof in a buffer appropriate for use in the determination method. Any buffer can be used so long as it does not interfere with ligand-receptor binding, such buffers including a phosphate buffer or a Tris-HCl buffer having pH of 4 to 10 (preferably pH of 6 to 8). For the purpose of minimizing non-specific binding, a surfactant such as CHAPS, Tween-80TM (manufactured by Kao-Atlas Inc.), digitonin or deoxycholate, and various proteins such as bovine serum albumin or gelatin, may optionally be added to the buffer. Further for the purpose of suppressing the degradation of the receptor or ligand by a protease, a protease inhibitor such as PMSF, leupeptin, E-64 (manufactured by Peptide Institute, Inc.) and pepstatin

may also be added. A given amount (5,000 to 500,000 cpm) of the test compound labeled with [^3H], [^{125}I], [^{14}C], [^{35}S] or the like is added to 0.01 ml to 10 ml of the receptor solution. To determine the amount of non-specific binding (NSB), a reaction tube containing an unlabeled test compound in a large excess is also provided. The reaction is carried out at approximately 0 to 50°C, preferably about 4 to 37°C for about 20 minutes to about 24 hours, preferably about 30 minutes to 3 hours. After completion of the reaction, the reaction mixture is filtrated through glass fiber filter paper, etc. and washed with an appropriate volume of the same buffer. The residual radioactivity in the glass fiber filter paper is then measured by means of a liquid scintillation counter or γ -counter. A test compound exceeding 0 cpm in count obtained by subtracting nonspecific binding (NSB) from the total binding (B) (B minus NSB) may be selected as a ligand (agonist) to the receptor protein of the present invention.

The method (4) or (5) above for determination of a ligand to the receptor protein of the present invention can be performed as follows. The receptor protein-mediated cell-stimulating activities (e.g., the activities that promote or suppress arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP production, intracellular cGMP production, inositol phosphate production, change in cell membrane potential, phosphorylation of intracellular proteins, activation of c-fos, pH reduction, etc.) may be determined by a publicly known method, or using an assay kit commercially available. Specifically, cells containing the receptor protein are first cultured on a multi-well plate, etc. Prior to the ligand determination, the medium is replaced with fresh

medium or with an appropriate non-cytotoxic buffer, followed by incubation for a given period of time in the presence of a test compound, etc. Subsequently, the cells are extracted or the supernatant is recovered and the resulting product is quantified by appropriate procedures. Where it is difficult to detect the production of the index substance for the cell-stimulating activity (e.g., arachidonic acid) due to a degrading enzyme contained in the cells, an inhibitor against such a degrading enzyme may be added prior to the assay. For detecting activities such as the cAMP production suppression activity, the baseline production in the cells is increased by forskolin or the like and the suppressing effect on the increased baseline production can then be detected.

The kit of the present invention for determination of the ligand that binds to the receptor protein of the present invention comprises the receptor protein of the present invention, cells containing the receptor protein of the present invention, or the membrane fraction of the cells containing the receptor protein of the present invention.

Examples of the ligand determination kit of the present invention are given below.

1. Reagents for determining ligands

(1) Buffers for assay and washing

Hanks' Balanced Salt Solution (manufactured by Gibco Co.) supplemented with 0.05% bovine serum albumin (Sigma Co.).

The solution is sterilized by filtration through a 0.45 μ m filter and stored at 4°C. Alternatively, the solution may be prepared at use.

(2) Standard G protein receptor protein

CHO cells on which the receptor protein of the present invention has been expressed are subjected to

The ligands that bind to the receptor protein of the present invention include substances specifically present in the brain, pituitary gland and pancreas. Examples of such ligands are angiotensin, bombesin, canavinoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioids, purines, vasopressin, oxytocin, PACAP, secretin, glucagon, calcitonin, adrenomedulin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, VIP (vasoactive intestinal peptide), somatostatin, dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene-related peptide), leukotriens, pancreastatin, prostaglandins, thromboxane, adenosine, adrenaline, α and β -chemokines (e.g. IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1 α , MIP-1 β , RANTES, etc.), endothelin, enterogastrin, histamine, neurotensin, TRH, pancreatic polypeptide, galanin, etc.

In the specification and drawings, the codes of bases and amino acids are denoted in accordance with the IUPAC-IUB Commission on Biochemical Nomenclature or by the common codes in the art, examples of which are shown below. For amino acids that may have the optical isomer, L form is presented unless otherwise indicated.

DNA : deoxyribonucleic acid
 cDNA : complementary deoxyribonucleic acid
 A : adenine
 T : thymine
 G : guanine
 C : cytosine
 I : inosine
 R : adenine (A) or guanine (G)
 Y : thymine (T) or cytosine (C)
 M : adenine (A) or cytosine (C)
 K : guanine (G) or thymine (T)
 S : guanine (G) or cytosine (C)

W : adenine (A) or thymine (T)
 B : guanine (G), guanine (G) or thymine (T)
 D : adenine (A), guanine (G) or thymine (T)
 V : adenine (A), guanine (G) or cytosine (C)
 5 N : adenine (A), guanine (G), cytosine (C) or
 thymine (T), or unknown or other base
 RNA : ribonucleic acid
 mRNA : messenger ribonucleic acid
 dATP : deoxyadenosine triphosphate
 10 dTTP : deoxythymidine triphosphate
 dGTP : deoxyguanosine triphosphate
 dCTP : deoxycytidine triphosphate
 ATP : adenosine triphosphate
 EDTA : ethylenediaminetetraacetic acid
 15 SDS : sodium dodecyl sulfate
 BHA : benzhydramine
 PMBHA: p-methyobenzhydramine
 Tos : p-toluenesulfonyl
 Bzl : benzyl
 20 Bom : benzyloxymethyl
 Boc : t-butyloxycarbonyl
 DCM : dichloromethane
 HOBt : 1-hydroxybenztriazole
 DCC : N,N'-dicyclohexylcarbodiimide
 25 TFA : trifluoroacetic acid
 DIEA : diisopropylethylamine
 Gly : glycine
 Ala : alanine
 Val : valine
 30 Leu : leucine
 Ile : isoleucine
 Ser : serine
 Thr : threonine
 Cys : cysteine
 35 Met : methionine

This shows the base sequence of primer R5 used in Example 1 later described.

[SEQ ID NO:7]

5 This shows the base sequence of primer hR1 used in Example 3 later described.

[SEQ ID NO:8]

This shows the amino acid sequence of the polypeptide (human type) of the present invention obtained in Example 3 later described.

10 [SEQ ID NO:9]

This shows the base sequence of DNA encoding the polypeptide of the present invention represented by SEQ ID NO:8.

[SEQ ID NO:10]

15 This shows the base sequence of primer bF6 used in Example 4 later described.

[SEQ ID NO:11]

This shows the base sequence of primer bF7 used in Example 4 later described.

20 [SEQ ID NO:12]

This shows the base sequence of primer bR6 used in Example 4 later described.

[SEQ ID NO:13]

25 This shows the base sequence of primer bR7 used in Example 4 later described.

[SEQ ID NO:14]

This shows the amino acid sequence of the polypeptide (bovine type) obtained in Example 4, which will be later described.

30 [SEQ ID NO:15]

This shows the base sequence of the DNA encoding the polypeptide of the present invention shown by SEQ ID NO:14.

[SEQ ID NO:16]

Agency of Industrial Science and Technology, National
 Institute of Bioscience and Human Technology (NIBH) as
 the Accession Number FERM BP-6558 on November 2, 1998
 and with Institute for Fermentation, Osaka (IFO) as the
 5 Accession Number IFO 16211 on October 16, 1998.

Escherichia coli transformant JM109/pbRF2 obtained
 in Example 9 later described was on deposit with the
 Ministry of International Trade and Industry, Agency of
 Industrial Science and Technology, National Institute
 10 of Bioscience and Human Technology (NIBH) as the
 Accession Number FERM BP-6811 on August 2, 1999 and
 with Institute for Fermentation, Osaka (IFO) as the
 Accession Number IFO 16288 on June 18, 1999.

Escherichia coli transformant JM109/phRF2 obtained
 15 in Example 8 later described was on deposit with the
 Ministry of International Trade and Industry, Agency of
 Industrial Science and Technology, National Institute
 of Bioscience and Human Technology (NIBH) as the
 Accession Number FERM BP-6812 on August 2, 1999 and
 20 with Institute for Fermentation, Osaka (IFO) as the
 Accession Number IFO 16289 on June 18, 1999.

Escherichia coli transformant JM109/pmLP4 obtained
 in Example 6 later described was on deposit with the
 Ministry of International Trade and Industry, Agency of
 25 Industrial Science and Technology, National Institute
 of Bioscience and Human Technology (NIBH) as the
 Accession Number FERM BP-6813 on August 2, 1999 and
 with Institute for Fermentation, Osaka (IFO) as the
 Accession Number IFO 16290 on June 18, 1999.

Escherichia coli transformant JM109/prLPL6
 30 obtained in Example 5 later described was on deposit
 with the Ministry of International Trade and Industry,
 Agency of Industrial Science and Technology, National
 Institute of Bioscience and Human Technology (NIBH) as
 35 the Accession Number FERM BP-6814 on August 2, 1999 and

with Institute for Fermentation, Osaka (IFO) as the
Accession Number IFO 16291 on June 18, 1999.

Escherichia coli transformant DH5 α /pCR2.1-h0T022T
obtained in Example 11 later described was on deposit
5 with the Ministry of International Trade and Industry,
Agency of Industrial Science and Technology, National
Institute of Bioscience and Human Technology (NIBH) as
the Accession Number FERM BP-6930 on November 8, 1999
and with Institute for Fermentation, Osaka (IFO) as the
10 Accession Number IFO 16330 on October 27, 1999.

Escherichia coli transformant DH5 α /pCR2.1-h0T022G
obtained in Example 11 later described was on deposit
with the Ministry of International Trade and Industry,
Agency of Industrial Science and Technology, National
15 Institute of Bioscience and Human Technology (NIBH) as
the Accession Number FERM BP-6931 on November 8, 1999
and with Institute for Fermentation, Osaka (IFO) as the
Accession Number IFO 16331 on October 27, 1999.

20 EXAMPLES

The present invention is described in detail below
with reference to Examples, but not intended to limit
the scope of the present invention thereto. The gene
manipulation procedures using *Escherichia coli* were
25 performed according to the methods described in the
Molecular Cloning.

Example 1 Synthesis of cDNA from human fetal brain
poly(A)⁺RNA fraction and amplification of
30 physiologically active peptide cDNA by RT-PCR

Oligo dT primer (Gibco BRL Inc.) was added as a
primer to 1 μ g of human fetal brain poly(A)⁺RNA
fraction available from Clontech and cDNA was
synthesized with reverse transcriptase from Moloney
35 murine leukemia virus (Gibco BRL Inc.) using a buffer

attached thereto. After completion of the reaction, the product was extracted with phenol : chloroform (1:1) and the extract was precipitated with ethanol. The precipitate was dissolved in 30 μ l of TE. Using a 1 μ l aliquot of the thus prepared cDNA as a template, amplification was performed by PCR using the following two primers (F5 and F6).

F5: 5'-GGGCTGCACATAGAGACTTAATTTTAG-3' (SEQ ID NO:3)
 10 F6: 5'-CTAGACCACCTCTATATAACTGCCCAT-3' (SEQ ID NO:4)

The reaction solution was composed of 20 pM each of the synthetic DNA primers (F5 and F6), 0.25 mM dNTPs, 0.5 μ l of Ex Taq DNA polymerase and 5 μ l of a buffer attached to the enzyme, which were mixed together to make the total volume of the reaction solution 50 μ l. Using Thermal Cycler (Perkin-Elmer Co.) for amplification, one cycle was set to include 98°C for 10 seconds, 63°C for 20 seconds and 72°C for 40 seconds. This cycle was repeated 40 times in total.

Using a 1 μ l aliquot of the PCR product as a template, the following two primers (F1 and R5) were amplified by nested PCR.

25 F1: 5'-GCACATAGAGACTTAATTTTAGATTAGAC-3' (SEQ ID NO:5)
 R5: 5'-CATGCACTTTGACTGGTTTCCAGGTAT-3' (SEQ ID NO:6)

The reaction solution was composed of 20 pM each of the synthetic DNA primers (F1 and R5), 0.25 mM dNTPs, 0.5 μ l of Ex Taq DNA polymerase and 5 μ l of a buffer attached to the enzyme, which were mixed together to make the total volume of the reaction solution 50 μ l. Using Thermal Cycler (Perkin-Elmer Co.) for amplification, one cycle was set to include 98°C for 10 seconds, 60°C for 20 seconds and 72°C for 40 seconds.

This cycle was repeated 40 times in total. The amplification product was confirmed by 1.2% agarose electrophoresis and ethidium bromide staining.

- 5 Example 2 Subcloning of the PCR products into plasmid vectors and selection of novel physiologically active peptide candidate clone by decoding base sequence of the inserted cDNA region

10 The PCR products obtained after the PCR procedure in Example 1 were separated by using a 1.2% agarose gel. After DNA fragments were proven to be amplified to the desired size, the DNAs were recovered using Quiagen PCR purification kit (Quiagen). According to the protocol attached to TA Cloning Kit (Invitrogen Co.), the
15 recovered DNAs were subcloned to plasmid vector pCRTM2.1. The recombinant vectors were introduced into *Escherichia coli* JM109 competent cells (Takara Shuzo Co., Ltd.) for transformation. Then, the resulting transformant clones bearing a cDNA-inserted fragment
20 were selected in an LB agar culture medium supplemented with ampicillin, IPTG and X-gal. Only transformant clones that showed white color were picked up with a sterilized toothpick to obtain transformant *Escherichia coli* JM109/phRF1.

25 After the individual clones were cultured overnight in an LB culture medium containing ampicillin, the clones were treated with an automated plasmid extracting machine (Kurabo Co., Ltd.) to prepare plasmid DNAs. An aliquot of the DNAs thus prepared was
30 cleaved by EcoRI to confirm the size of the cDNA fragment inserted. An aliquot of the remaining DNAs was further treated with RNase, extracted with phenol/chloroform followed by concentrating the aliquot through ethanol precipitation. Sequencing was carried
35 out by using DyeDeoxy Terminator Cycle Sequencing Kit

(ABI Inc.), the DNAs were decoded using an automated fluorescent sequencer. The data of the base sequences obtained were read by DNASIS (Hitachi System Engineering Co., Ltd.). The base sequence determined is shown in FIG. 1.

The base sequence thus determined was subjected to homology retrieval and sequence analysis based on FIG. 1. The results reveal that the novel physiologically active peptide was encoded by the cDNA fragment inserted in the plasmid of the transformant *Escherichia coli* JM109/phRF1.

Example 3 Acquisition of splicing variant of the physiologically active peptide cDNA from human fetal brain cDNA

Using as a template 1 ml of the human fetal brain cDNA prepared in Example 1, amplification was performed by PCR using the following two primers (F5 and hR1).

F5: 5'-GGGCTGCACATAGAGACTTAATTTTAG-3' (SEQ ID NO:3)
hR1: 5'-CAGCTTTAGGGACAGGCTCCAGGTTTC-3' (SEQ ID NO:7)

The reaction solution was composed of 20 pM each of the synthetic DNA primers (F5 and hR1), 0.25 mM dNTPs, 0.5 ml of Ex Taq DNA polymerase and a buffer attached to the enzyme, which were mixed together to make the total volume of the reaction solution 50 ml. Using Thermal Cycler (Perkin-Elmer Co.) for amplification, one cycle was set to include 98°C for 10 seconds, 65°C for 20 seconds and 72°C for 20 seconds. This cycle was repeated 40 times in total. The amplification product was confirmed by 1.2% agarose electrophoresis and ethidium bromide staining. After the PCR product was proven to be amplified, the reaction product was purified using QUIA Quick PCR

buffer attached to the enzyme was made the total volume of the reaction solution 25 ml. Using Thermal Cyclor (Perkin-Elmer Co.) for amplification, one cycle was set to include 98°C for 10 seconds and 72°C for 2 minutes, which cycle was repeated 5 times, further cycle was set to include 98°C for 10 seconds and 70°C for 2 minutes, which cycle was repeated 5 times, and another cycle set to include 98°C for 10 seconds and 68°C for 2 minutes and 30 seconds, which cycle was repeated 25 times. Then, the reaction solution of the first PCR was diluted to 10-fold, 1 ml of the aliquot was used as a template to perform a second PCR using (bF7 and AP2) as primers. The reaction solution composed of 20 pM each of the primers, 0.25 mM dNTPs, 0.5 ml of Klen Taq DNA polymerase and a buffer attached to the enzyme was made the total volume of the reaction solution 25 ml. Using a Thermal Cyclor (Perkin-Elmer Co.) for amplification, one cycle was set to include 98°C for 10 seconds and 72°C for 2 minutes, which cycle was repeated 5 times, followed by another cycle set to include 98°C for 10 seconds and 70°C for 2 minutes, which cycle was repeated 5 times and then a further cycle set to 98°C for 10 seconds and 68°C for 2 minutes and 30 seconds, which cycle was repeated 35 times.

For amplification of the 3' terminus (C-terminal region), a first PCR was carried out using the synthetic primers (bF6 and AP1). The reaction solution composed of 20 pM each of the primers, 0.25 mM dNTPs, 0.5 ml of Klen Taq DNA polymerase and a buffer attached to the enzyme was made the total volume of the reaction solution 25 ml. Using Thermal Cyclor (Perkin-Elmer Co.) for amplification, one cycle was set to include 98°C for 10 seconds and 72°C for 2 minutes, which cycle was repeated 5 times, and another cycle set to include 98°C for 10 seconds and 70°C for 2 minutes, which cycle was

repeated 5 times and a further cycle set to include 98°C for 10 seconds and 68°C for 2 minutes and 30 seconds, which cycle was repeated 25 times. Then, the reaction solution of the first PCR was diluted to 10-fold, 1 ml of the aliquot was used as a template to perform a second PCR using (bF7 and AP2) as primers. The reaction solution composed of 20 pM each of the primers, 0.25 mM dNTPs, 0.5 ml of Klen Taq DNA polymerase and a buffer attached to the enzyme was made the total volume of the reaction solution 25 ml. Using Thermal Cycler (Perkin-Elmer Co.) for amplification, one cycle was set to include 98°C for 10 seconds and 72°C for 2 minutes, which cycle was repeated 5 times, followed by another cycle set to include 98°C for 10 seconds and 70°C for 2 minutes, which cycle was repeated 5 times and then a further cycle set to 98°C for 10 seconds and 68°C for 2 minutes and 30 seconds, which cycle was repeated 35 times. The amplification products at the 5' and 3' termini were confirmed by 1.2% agarose gel electrophoresis and ethidium bromide staining, respectively. After the PCR product was confirmed to be amplified, the reaction product was purified using QIA quick PCR purification Kit (Quiagen), followed by sequencing. The sequencing reaction was conducted using BigDye Deoxy Terminator Cycle Sequence Kit (ABI). The DNAs were decoded using an automated fluorescent sequencer (ABI377).

The data of the base sequences obtained were read by DNASIS (Hitachi System Engineering Co., Ltd.). The base sequence determined (SEQ ID NO:15) and the deduced amino acid sequence (SEQ ID NO:14) are shown in FIG. 4.

Example 5 Acquisition of physiologically active peptide cDNA from rat brain poly(A)⁺RNA

Rat type physiologically active peptide cDNA was

obtained from rat brain poly(A)⁺RNA using Marathon cDNA Amplification Kit (Clontech). Using as a template rat brain cDNA prepared in accordance with the manual attached to the Kit, the following two primers were synthesized and employed in combination with two primers AP1 and AP2 attached to the Kit to effect amplification by PCR.

10 rLPR1: 5'-CCCTGGGGCTTCTTCTGTCTTCTATGT-3' (SEQ ID NO:16)

rLPF1: 5'-AGCGATTCATTTTATTGACTTTAGCA-3' (SEQ ID NO:17)

For amplification of the 5' terminus (N-terminal region), a first PCR was carried out using the primer set of rLPR1 and AP1. The reaction solution composed of 20 pM each of the primers, 0.1 mM dNTPs, 0.25 ml of Klen Taq DNA polymerase was made the total volume of the reaction solution 25 ml with a buffer attached to the enzyme. Using Thermal Cycler (Perkin-Elmer Co.) for amplification, one cycle was set to include 98°C for 10 seconds and 72°C for 2 minutes, which cycle was repeated 5 times, another cycle set to include 98°C for 10 seconds and 70°C for 2 minutes, which cycle was repeated 5 times and then a further cycle set to include 98°C for 10 seconds and 68°C for 2 minutes and 30 seconds, which cycle was repeated 25 times. Then, a second PCR was performed using the first PCR solution as a template, the first set of primers and the same compositions of the reaction solution. For amplification, one cycle was set to include 98°C for 10 seconds and 72°C for 2 minutes, which cycle was repeated 5 times, followed by another cycle set to include 98°C for 10 seconds and 70°C for 2 minutes, which cycle was repeated 5 times and then a further cycle set to 98°C

for 10 seconds (68°C for 2 minutes and 30 seconds),
which cycle was repeated 38 times.

For amplification of the 3' terminus (C-terminal
region), a first PCR was carried out using the primer
set of rLPF1 and AP1. The composition of the reaction
solution was the same as that for amplification of the
5'-terminus (N-terminal region). For amplification, one
cycle was set to include 98°C for 10 seconds and 72°C
for 2 minutes, which cycle was repeated 5 times,
another cycle set to include 98°C for 10 seconds and
72°C for 2 minutes, which cycle was repeated 5 times
and a further cycle set to include 98°C for 10 seconds,
65°C for 20 seconds and 72°C for 2 minutes, which cycle
was repeated 25 times. Then, the reaction solution of
the first PCR was used as a template to perform a
second PCR using rLPF1 and AP2 primers. The composition
of the reaction solution was the same as that for the
first PCR. Using Thermal Cyclor (Perkin-Elmer Co.) for
amplification, one cycle was set to include 98°C for 10
seconds and 72°C for 2 minutes, which cycle was
repeated 5 times, followed by another cycle set to
include 98°C for 10 seconds and 70°C for 2 minutes,
which cycle was repeated 5 times and then a further
cycle set to 98°C for 10 seconds, 65°C for 20 seconds
and 72°C for 2 minutes, which cycle was repeated 38
times. The amplification products at the 5' and 3'
termini were confirmed by 1.2% agarose gel
electrophoresis and ethidium bromide staining,
respectively. The PCR product band was purified using
QIA quick Gel Extrication Kit (Quiagen), followed by
sequencing. The sequencing was conducted in a manner
similar to Example 3. The base sequence determined (SEQ
ID NO:19) and the deduced amino acid sequence (SEQ ID
NO:18) are shown in FIG. 5. Based on the sequences, two

200 pM each of the primers, 0.1 mM dNTP, 0.25 ml of Klen Taq DNA polymerase was made the total volume of the reaction solution 25 ml with a buffer attached to the enzyme. For amplification, one cycle was set to include 98°C for 10 seconds and 72°C for 2 minutes, which cycle was repeated 5 times, another cycle set to include 98°C for 10 seconds and 70°C for 2 minutes, which cycle was repeated 5 times and a further cycle set to include 98°C for 10 seconds and 68°C for 2 minutes and 30 seconds, which cycle was repeated 25 times. Then, the reaction solution of the first PCR was used as a template to perform a second PCR. Amplification at the 5' terminus was performed using the same primer set as in the first PCR and for amplification at the 3' terminus, the same composition of the reaction solution as in the first PCR was prepared, using the primer set of mF3 and AP1. PCR was carried out by repeating 5 times a cycle set to include 98°C for 10 seconds and 72°C for 2 minutes, 5 times another cycle set to include 98°C for 10 seconds and 70°C for 2 minutes, and then 38 times a further cycle set to 98°C for 10 seconds and 68°C for 2 minutes and 30 seconds.

The amplification products at the 5' and 3' termini were confirmed by 1.2% agarose gel electrophoresis and ethidium bromide staining, respectively. The PCR product band was purified using QIA quick Gel Extrication Kit (Quiagen), followed by sequencing. The sequencing was conducted in a manner similar to Example 3.

Based on the sequences, two primers were further synthesized.

moF: 5'-TTTAGACTTAGACGAAATGGA-3' (SEQ ID NO:31)
moR: 5'-GCTCCGTAGCCTCTTGAAGTC-3' (SEQ ID NO:32)

Using as a template the above-described cDNA that was synthesized from mouse brain poly(A)⁺ RNA using SuperScript II RNase H-reverse, PCR was carried out to amplify a fragment containing mouse physiologically active peptide full-length cDNA. The reaction was carried out using Klen Taq DNA polymerase (Clontech), by repeating 35 times a cycle set to include 98°C for 10 seconds, 56°C for 20 seconds and 72°C for 15 seconds. The amplification product of about 600 bp was confirmed by 2% agarose electrophoresis and ethidium bromide staining. The band was purified using QIA quick Gel Extrication Kit (Quiagen), subcloned to cloning vector pCR2.1-TOPO (TOPO TA cloning kit, Invitrogen Inc.) and then introduced into *Escherichia coli* JM109 to obtain transformant E. coli JM109/pmLP4. The base sequence was determined in a manner similar to Example 3. The base sequence thus determined (SEQ ID NO:34) and the deduced amino acid sequence (SEQ ID NO:33) therefrom are shown in FIG. 7.

Example 7

(1) Cloning of the cDNA encoding the rat cerebellum-derived G protein-coupled receptor protein and determination of the base sequence

Using rat cerebellum-derived cDNA as a template and two primers, namely, primer 1 (SEQ ID NO :35) and primer 2 (SEQ ID NO :36), PCR was carried out. The reaction solution in the above reaction comprised of 1/10 volume of the cDNA, 1/50 volume of Advantage cDNA Polymerase Mix (CLONTEC Inc.), 0.2 µM of primer 1 (SEQ ID NO :35), 0.2 µM of primer 2 (SEQ ID NO :36), 200 µM dNTPs and a buffer attached to the enzyme to make the final volume 50 µl. The PCR was carried out by cycles of (1) 94°C for 2 minutes, (2) then a cycle set to

include 94°C for 30 seconds followed by 72°C for 2 minutes, which was repeated 3 times, (3) a cycle set to include 94°C for 30 seconds followed by 68°C for 2 minutes, which was repeated 3 times, (4) a cycle set to include 94°C for 30 seconds followed by 64°C for 30 seconds and 68°C for 2 minutes, which was repeated 30 times, and (5) finally, extension reaction at 68°C for 8 minutes. After completion of the PCR reaction, the product was subcloned to plasmid vector pCR2.1 (Invitrogen Inc.) following the instructions attached to the TA cloning kit (Invitrogen Inc.), which was then introduced into *Escherichia coli* DH5 α , and the clones containing the cDNA were selected on LB agar plates containing ampicillin. The sequence of each clone was analyzed to give the cDNA sequence (SEQ ID NO :38) encoding the novel G protein-coupled receptor protein. The novel G protein-coupled receptor protein containing the amino acid sequence (SEQ ID NO :1) deduced therefrom was designated rOT7T022L.

Plasmid pAK-rOT7T022L in which the cDNA (SEQ ID NO:38) encoding the rat cerebellum-derived G protein-coupled receptor protein rOT7T022L of the present invention was subcloned was introduced into *Escherichia coli* DH10B according to a publicly known method to give transformant *Escherichia coli* DH10B/pAK-rOT7T022L.

(2) Establishment of G protein-coupled receptor protein rOT7T022L-expressing CHO cell

CHOdhr⁻ cells of 1 x 10⁶ were inoculated on Petri's dish of a 10 mm diameter for tissue culture followed by incubation for 24 hours. Using 20 μ g of rOT7T022L-expressing vector pAK-rOT7T022L obtained (1), DNA-liposome complex was formed by the liposome method using a gene transfer kit (Gene Transfer, Nippon Gene Co.). After a fresh medium was exchanged for the medium,

the DNA-liposome complex was added to the medium and incubated overnight. The medium was replaced with a fresh medium and further incubation was performed for one day followed by incubation for 2 days for transformant selection. The cells in the Petri's dish were recovered by treatment with trypsin-EDTA. By culturing again in a dilute cell density, the ratio of transformants was increased thereby to obtain stable clone of cell line CHO-rOT7T022L capable of expressing rOT7T022L in a high level.

(3) Synthesis of Met-Pro-His-Ser-Phe-Ala-Asn-Leu-Pro-Leu-Arg-Phe-NH₂ (SEQ ID NO:39)

Commercially available p-methyl BHA resin, 0.5 mmole, (manufactured by Applied Biosystems, now Perkin-Elmer Inc.) was charged in a reaction tank of peptide synthesizer (430A manufactured by Applied Biosystems). After swelling with DCM, first amino acid Boc-Phe was activated with the HOBt/DCC method and then introduced into p-methyl BHA resin. The resin was treated with 50% TFA/DCM to remove Boc, wherein the amino group was liberated and neutralized with DIEA. Next amino acid Boc-Arg(Tos) was condensed to the amino group by the HOBt/DCC method. Ninhydrin test was conducted to examine if any unreacted amino group was present. After it was confirmed that the reaction was completed, Boc-Leu, Boc-Pro, Boc-Leu, Boc-Asn, Boc-Ala, Boc-Phe, Boc-Ser(Bzl), Boc-His(Bom), Boc-Pro and Boc-Met were introduced in this order. The resin in which all amino acids of the sequence were introduced was treated with 50% TFA/DCM to remove the Boc groups on the resin. Thereafter the resin was dried to give 0.73 g of Met-Pro-His(Bom)-Ser(Bzl)-Phe-Ala-Asn-Leu-Pro-Leu-Arg(Tos)-Phe-pMBHA-resin.

In a Teflon-made hydrogen fluoride reactor the resin, 0.25 g, was reacted in 15 ml of hydrogen

fluoride together with 5.1 g of p-cresol at 0°C for 60 minutes. After removing the hydrogen fluoride by distillation in vacuum, 100 ml of diethyl ether was added to the residue, stirred and filtrated through a glass filter followed by drying. The dried product was suspended in 50 ml of 50% acetic acid aqueous solution and stirred. After the peptide was extracted, it was separated from the resin and concentrated to about 5 ml in vacuum. The concentrate was applied to a column of Sephadex G-25 (2 x 90 cm) and developed with 50% acetic acid aqueous solution. Main fractions were collected and lyophilized. Next, the crudely purified peptide was dissolved in 1.5 ml of 5% thioglycolic acid/50% acetic acid. The solution was kept at 50°C for 12 hours to reduce the Met-oxidized peptide. The peptide was applied to a reversed phase column filled up with LiChroprep (trade name) RP-18 (manufactured by MERCK Inc.) followed by repeating purification with gradient elution using 0.1% aqueous TFA and 33% acetonitrile aqueous solution containing 0.1% TFA. Fractions eluted at the acetonitrile concentration of about 27% were collected and lyophilized to give 26 mg of white powders.

Mass spectrum (M+H)⁺ 1428.7 (calcd. 1428.8)

Elution time on HPLC: 18.0 mins.

Column conditions:

Column: Wakosil (trademark) 5C18 (4.6 x 100 mm)

Eluant: linear density gradient elution (25 mins.)

with solution A to solution B, using solution A

(5% aqueous acetonitrile solution containing

0.1% TFA) and solution B (55% aqueous

acetonitrile solution containing 0.1% TFA)

Flow rate: 1.0 ml/min.

Example 8 Construction of transformant bearing splicing variant cDNA for human novel physiologically active peptide candidate

The reaction product obtained after PCR in Example 3 supra was separated using 1.2% agarose gel. After DNA fragments were proven to be amplified to the desired size, the DNAs were recovered using Quiagen PCR purification kit (Quiagen). According to the protocol attached to TA Cloning Kit (Invitrogen Co.), the recovered DNAs were subcloned to plasmid vector pCRTM2.1. The recombinant vectors were introduced into *Escherichia coli* JM109 competent cells (Takara Shuzo Co.) for transformation. Then, the resulting clones bearing the cDNA-inserted fragment were selected in an LB agar culture medium supplemented with ampicillin, IPTG and X-gal. Only transformant clones that showed white color were picked up with a sterilized toothpick. Each clone was cultured overnight in an LB culture medium supplemented with ampicillin and plasmid DNA was prepared using an automated plasmid extracting machine (Kurabo Co., Ltd.). An aliquot of the DNAs thus prepared was cleaved by EcoRI to confirm the size of the cDNA fragment inserted. An aliquot of the remaining DNAs was further treated with RNase, extracted with phenol/chloroform followed by concentrating the aliquot through ethanol precipitation. The reaction for sequencing was carried out by using DyeDeoxy Terminator Cycle Sequencing Kit (ABI Inc.), the DNAs were decoded using an automated fluorescent sequencer to obtain transformant *Escherichia coli* JM109/phRF2.

Example 9 Construction of transformant bovine novel physiologically active peptide cDNA

Using as a template 1 ml of the bovine hypothalamus cDNA prepared in Example 4, amplification

was performed by PCR using the following two primers (bFF and bFR).

bFF: 5'-TTCTAGATTTTGGACAAAATGGAAATT-3' (SEQ ID NO:52)

5 bFR: 5'-CGTCTTTAGGGACAGGCTCCAGATTTC-3' (SEQ ID NO:53)

The reaction solution was composed of 20 pM each of the synthetic primers (bFF and bFR), 0.25 mM dNTPs, 0.5 ml of Ex Taq DNA polymerase and a buffer attached to the enzyme, which were mixed together to make the total volume 50 ml. Using Thermal Cycler (Perkin-Elmer Co.) for amplification, one cycle was set to include 98°C for 10 seconds, 65°C for 20 seconds and 72°C for 20 seconds, which cycle was repeated 40 times. The amplification product was confirmed by 1.2% agarose electrophoresis and ethidium bromide staining. The reaction product obtained after PCR in Example 3 was separated using 1.2% agarose gel. After the DNA fragments were proven to be amplified to the desired size, the DNAs were recovered using Quigen PCR Purification Kit (Quiagen). According to the protocol attached to TA Cloning Kit (Invitrogen Co.), the recovered DNAs were subcloned to plasmid vector pCRTM2.1. The recombinant DNA was introduced into *Escherichia coli* JM109 competent cells (Takara Shuzo Co., Ltd.) for transformation. Then, the resulting clones bearing a cDNA-inserted fragment were selected in an LB agar culture medium supplemented with ampicillin, IPTG and X-gal. Only clones that showed white color were picked up with a sterilized toothpick. Each clone was cultured overnight in an LB culture medium supplemented with ampicillin and plasmid DNA was prepared using an automated plasmid extracting machine (Kurabo Co., Ltd.). An aliquot of the DNAs thus prepared was cleaved by EcoRI to confirm the size of

the cDNA fragment inserted. The DNAs prepared were further treated with RNase, extracted with phenol/chloroform and the extract was concentrated by ethanol precipitation. The reaction for sequencing was carried out by using DyeDeoxy Terminator Cycle Sequencing Kit (ABI Inc.), the DNAs were decoded using an automated fluorescent sequencer to obtain transformant *Escherichia coli* JM109/pbRF2.

Example 10 Activity of suppressing cAMP production of peptide MPHSFANLPLRFamide (SEQ ID NO:39) and peptide VPNLQRFamide (SEQ ID NO:40) for rOT7T022L(SEQ ID NO:37)-expressing CHO cells

It was confirmed by the site sensor experiment of Example 7 (6) that peptide MPHSFANLPLRFamide (SEQ ID NO:39) and peptide VPNLQRFamide (SEQ ID NO:40) synthesized in Example 7 (3) and (4) specifically reacted with the rOT7T022L receptor. Next, the cAMP production suppression activity of the peptides for the rOT7T022L-expressing CHO cells were evaluated.

The rOT7T022L-expressing CHO cells obtained in Example 7 (2) above was inoculated in a 24-well plate in a concentration of 1.0×10^5 cells/well, followed by incubation at 37°C for 2 days. After the cells were washed with Hanks' buffer (HBSS) supplemented with 0.05% BSA and 0.2 mM IBMX, the system was allowed to stand at 37°C for 30 minutes in the same buffer. Thirty minutes after, an assay buffer was prepared by adding the cells to Hanks' buffer supplemented with 10^{-6} M Forskolin and at the same time, the peptides described above were added thereto in various concentrations. Incubation was performed at 37°C for 30 minutes. According to the method given in cAMP EIA Kit (Amersham Inc.), the cAMP level in the cells of each well was measured 30 minutes after. As shown in FIG. 9, peptide

MPHSFANLPLRFamide (SEQ ID NO:39) and peptide
VPNLPQRFamide (SEQ ID NO:40) showed a potent effect of
cAMP production suppression on rOT7T022L receptor-
expressing CHO cells at IC₅₀ of 0.5 nM and 0.7 nM,
5 respectively, indicating that the peptide
concentrations were very low.

Example 11 Cloning of the cDNA encoding human
hypothalamus G protein-coupled receptor protein and
10 determination of its base sequence

Using human hypothalamus cDNA (CLONTECH Inc.) as a
template and two primers: primer 1, 5'-GTCGACATGG
AGGGGGAGCC CTCCCAGCCT C-3' (SEQ ID NO :57) and primer 2,
5'-ACTAGTTCAG ATATCCCAGG CTGGAATGG-3' (SEQ ID NO :58),
15 PCR was carried out. The reaction solution in the
above reaction comprised of 1/10 volume of the cDNA,
which was used as a template, 1/50 volume of Advantage
cDNA Polymerase Mix (CLONTECH Inc.), 0.2 µM of primer 1
(SEQ ID NO :57), 0.2 µM of primer 2 (SEQ ID NO :58),
20 200 µM dNTPs, 4% dimethylsulfoxide and a buffer
attached to the enzyme to make the final volume 25 µl.
The PCR was carried out by (1) a cycle of 94°C for 2
minutes, (2) then a cycle set to include 94°C for 20
seconds followed by 72°C for 1 minute and 30 seconds,
25 which was repeated 3 times, (3) a cycle set to include
94°C for 20 seconds followed by 67°C for 1 minute and 30
seconds, which was repeated 3 times, (4) a cycle set to
include 94°C for 20 seconds followed by 62°C for 20
seconds and 68°C for 1 minute and 30 seconds, which was
30 repeated 38 times, and (5) finally, extension reaction
at 68°C for 7 minutes. After completion of the PCR
reaction, the reaction product was subcloned to plasmid
vector pCR2.1 (Invitrogen Inc.) following the
instructions attached to the TA cloning kit (Invitrogen
35 Inc.), which was then introduced into *Escherichia coli*

DH5 α , and the clones carrying the cDNA were selected in an LB agar medium containing ampicillin. The sequence of each clone was analyzed to give the cDNA sequences (SEQ ID NO :55 and SEQ ID NO:56) encoding the novel G protein-coupled receptor protein. The two sequences are different by one base in the 597th residue but the deduced amino acid sequences are the same (SEQ ID NO:57). Novel G protein-coupled receptor protein containing the amino acid sequence was designated hOT7T022. The two transformants were named *Escherichia coli* DH5 α /pCR2.1-hOT022T (containing cDNA shown by SEQ ID NO:55) and *Escherichia coli* DH5 α /pCR2.1-hOT022G (containing cDNA shown by SEQ ID NO:56).

15 Industrial Applicability

The polypeptide, receptor protein, etc. of the present invention exhibits, e.g., a nerve cell stimulating activity and thus can be employed as a pharmaceutical composition for the treatment of neuropathy. The polypeptide or receptor protein of the present invention is useful as a reagent for screening a compound that accelerates or inhibits the activities of the polypeptide or receptor protein of the present invention, or its salts. These compounds obtained by the screening are expected to be useful as an agent for the treatment/prevention of neuropathy. Furthermore, antibodies to the polypeptide or receptor protein of the present invention can recognize the polypeptide or receptor protein of the present invention specifically and can be used for quantification of the polypeptide or receptor protein of the present invention in a test sample fluid.

What is claimed is:

1. A polypeptide containing the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO:1, its amide or ester, or a salt thereof.
2. A polypeptide or its amide or ester, or a salt thereof, according to claim 1, wherein substantially the same amino acid sequence is represented by SEQ ID NO:8, SEQ ID NO:14, SEQ ID NO:18, SEQ ID NO:33 or SEQ ID NO:50.
3. A partial peptide of the polypeptide according to claim 1, or its amide or ester, or a salt thereof.
4. A partial peptide or its amide or ester, or a salt thereof, according to claim 3, comprising amino acid residues 81 (Met) to 92 (Phe) of SEQ ID NO:1.
5. A partial peptide or its amide or ester, or a salt thereof, according to claim 3, comprising amino acid residues 101 (Ser) to 112 (Ser) of SEQ ID NO:1.
6. A partial peptide or its amide or ester, or a salt thereof, according to claim 3, comprising amino acid residues 124 (Val) to 131 (Phe) of SEQ ID NO:1.
7. An amide of the partial peptide of the polypeptide according to claim 1, or a salt thereof.
8. A DNA containing a DNA bearing a base sequence encoding the polypeptide of claim 1.
9. A DNA according to claim 8 having the base sequence represented by SEQ ID NO:2, SEQ ID NO:9, SEQ ID NO:15, SEQ ID NO:19, SEQ ID NO:34 or SEQ ID NO:51.
10. A DNA containing a DNA encoding the partial peptide of claim 3.
11. A DNA according to claim 10, comprising bases 241 to 276 of the base sequence represented by SEQ ID NO:2.

12. A DNA according to claim 10, comprising bases 301 to 336 of the base sequence represented by SEQ ID NO:2.

13. A DNA according to claim 10, comprising bases 370 to 393 of the base sequence represented by SEQ ID NO:2.

14. A recombinant vector containing the DNA of claim 8 or claim 10.

15. A transformant transformed with the recombinant vector of claim 14.

16. A method for manufacturing the polypeptide or its amide or ester, or a salt thereof, according to claim 1 or the partial peptide or its amide or ester, or a salt thereof, according to claim 3, which comprises culturing said transformant of claim 15 and producing and accumulating the polypeptide of claim 1 or the partial peptide of claim 3.

17. An antibody to the polypeptide or its amide or ester, or a salt thereof, according to claim 1 or the partial peptide or its amide or ester, or a salt thereof according to claim 3.

18. A diagnostic composition comprising the DNA according to claim 8 or claim 10 or the antibody according to claim 17.

19. An antisense DNA having a complementary or substantially complementary base sequence to the DNA according to claim 8 or claim 10 and capable of suppressing expression of said DNA.

20. A composition comprising the polypeptide or its amide or ester, or a salt thereof, according to claim 1 or the partial peptide, or its amide or ester, or a salt thereof, according to claim 3.

21. A pharmaceutical composition comprising the polypeptide or its amide or ester, or a salt thereof,

according to claim 1 or the partial peptide or its amide or ester, or a salt thereof, according to claim 3.

22. A method for screening a compound that accelerates or inhibits the activity of the polypeptide or its amide or ester, or a salt thereof, according to claim 1 or the partial peptide or its amide or ester, or a salt thereof, according to claim 3, which comprises using the polypeptide or its amide or ester, or a salt thereof, according to claim 1 or the partial peptide or its amide or ester, or a salt thereof, according to claim 3.

23. A method for screening according to claim 22, wherein the polypeptide or its amide or ester, or a salt thereof, according to claim 1 or the partial peptide or its amide or ester, or a salt thereof, according to claim 3 and a protein containing the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO:37, or a salt thereof, or the partial peptide or its amide or ester, or a salt thereof, are employed.

24. A kit for screening a compound that accelerates or inhibits the activity of the polypeptide or its amide or ester, or a salt thereof, according to claim 1 or the partial peptide or its amide or ester, or a salt thereof, according to claim 3, comprising the polypeptide or its amide or ester, or a salt thereof, according to claim 1, or the partial peptide or its amide or ester, or a salt thereof, according to claim 3.

25. A kit for screening according to claim 24, comprising the polypeptide or its amide or ester, or a salt thereof, according to claim 1 or the partial peptide or its amide or ester, or a salt thereof, according to claim 3 and a protein containing the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO:37 or the

partial peptide or its amide or ester, or a salt thereof.

26. A compound that accelerates or inhibits the polypeptide, or its amide or ester, or a salt thereof, according to claim 1 or the partial peptide, or its amide or ester, or a salt thereof, according to claim 3, which is obtainable using the screening method according to claim 22 or the screening kit according to claim 24.

27. A pharmaceutical composition comprising a compound that accelerates or inhibits the polypeptide, or its amide or ester, or a salt thereof, according to claim 1 or the partial peptide, or its amide or ester, or a salt thereof, according to claim 3, which is obtainable using the screening method according to claim 22 or the screening kit according to claim 24.

28. A protein or a salt thereof containing the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO:37.

29. A protein or a salt thereof according to claim 28, wherein substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO:37 is the amino acid sequence represented by SEQ ID NO:54.

30. A partial peptide or its amide or ester, or a salt thereof, according to claim 28.

31. A DNA containing a DNA having a base sequence encoding the protein according to claim 28 or the partial peptide according to claim 30.

32. A DNA according to claim 31 having the base sequence represented by SEQ ID NO:38, SEQ ID NO:55 or SEQ ID NO:56.

33. A recombinant vector containing the DNA according to claim 31.

34. A transformant transformed with the recombinant vector according to claim 33.

35. A method for manufacturing the protein or a salt thereof according to claim 28 or the partial peptide or its amide or ester, or a salt thereof, according to claim 30, which comprises culturing the transformant according to claim 34 and producing and accumulating the protein according to claim 28 or the partial peptide according to claim 30.

36. An antibody to the protein or a salt thereof according to claim 28 or the partial peptide or its amide or ester, or a salt thereof, according to claim 30.

37. A diagnostic composition comprising the DNA according to claim 31 or the antibody according to claim 36.

38. A ligand to the protein or a salt thereof according to claim 28, which is obtainable by using the protein or a salt thereof according to claim 28 or the partial peptide or its amide or ester or, a salt thereof, according to claim 30.

39. A method for determination of a ligand to the protein or a salt thereof according to claim 28, characterized by using the protein or a salt thereof according to claim 28 or the partial peptide or its amide or ester, or a salt thereof, according to claim 30.

40. A method for screening a compound that alters the binding property between a ligand and the protein or a salt thereof according to claim 28, which comprises using the protein or a salt thereof according to claim 28 or the partial peptide or its amide or ester, or a salt thereof, according to claim 30.

41. A kit for screening a compound that alters the binding property between a ligand and the protein or a salt thereof according to claim 28, comprising the protein or a salt thereof according to claim 28 or the

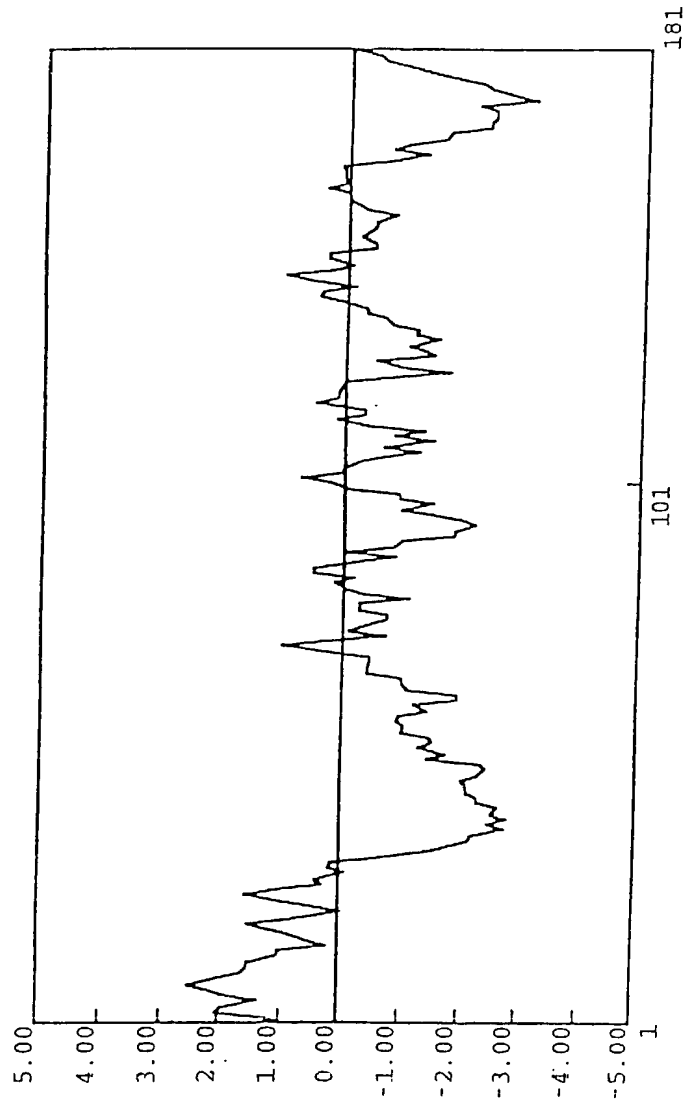
partial peptide or its amide or ester, or a salt thereof, according to claim 30.

42. A compound that alters the binding property between a ligand and the protein or a salt thereof according to claim 28, which is obtainable by using the screening method according to claim 40 or the screening kit according to claim 41.

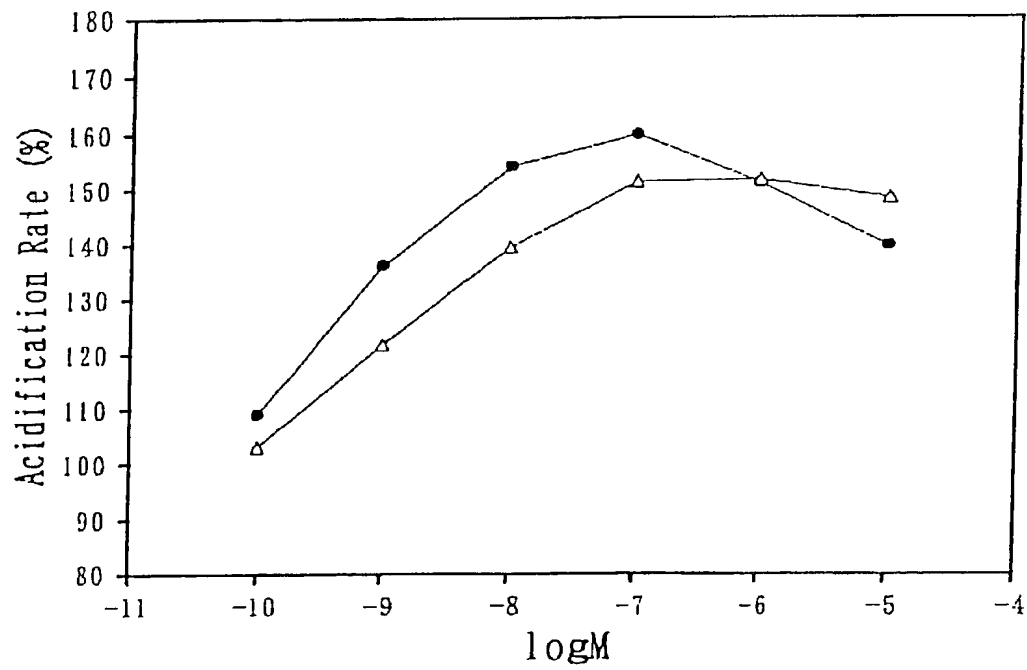
43. A pharmaceutical composition comprising a compound that alters the binding property between a ligand and the protein or a salt thereof according to claim 28, which is obtainable by using the screening method according to claim 40 or the screening kit according to claim 41.

44. A method for quantifying the protein or a salt thereof according to claim 28, which comprises using the antibody of claim 36.

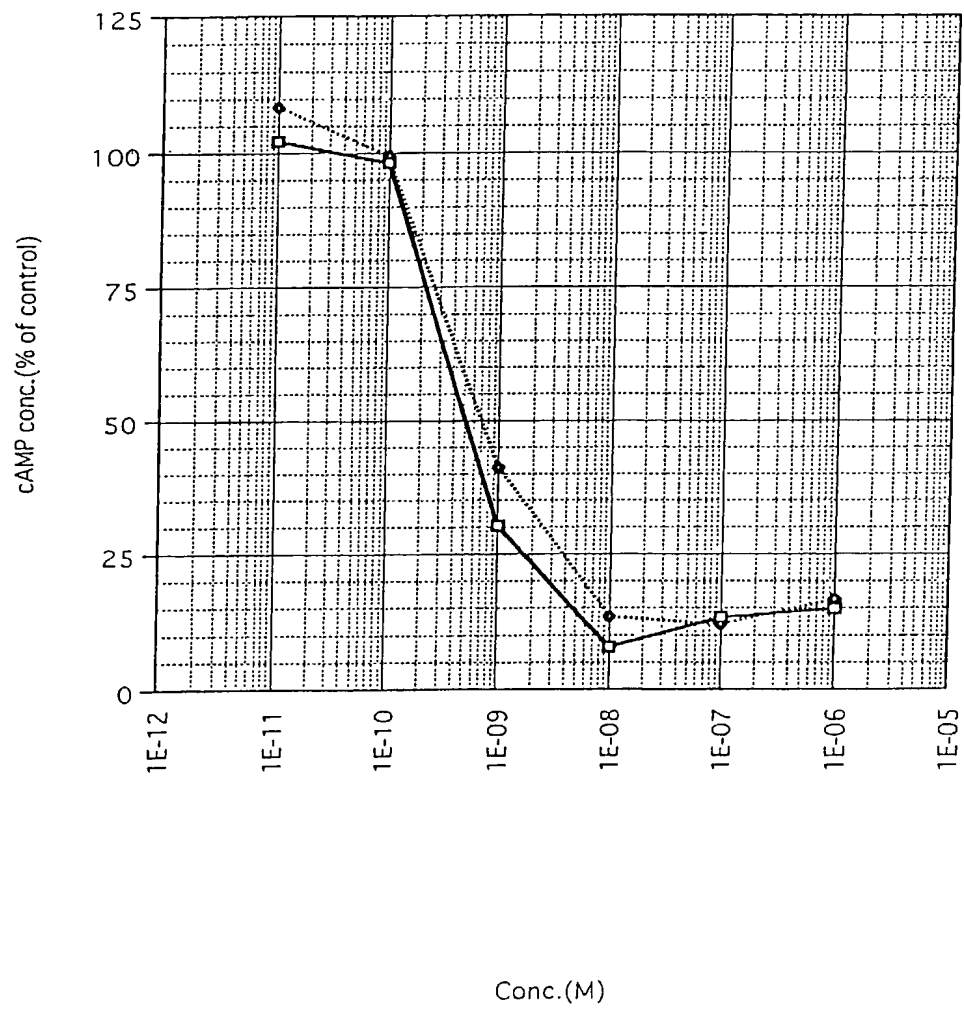
F i g . 2



F i g . 8



F i g . 9



1-6 200	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS		
		<u>Watanabe</u>	<u>Takuya</u>	
		<u>Osaka</u>	Japan <u>JPV</u>	Japan
	14-9-B904, Niitaka 6-chome, Yodogawa-ku, Osaka-shi, Osaka 532-0033 Japan			

2-6 202	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS		
		<u>Kikuchi</u>	<u>Kuniko</u>	
		<u>Ibaraki</u>	Japan <u>JPV</u>	Japan
	8-18-101, Shinmachi 5-chome, Toride-shi, Ibaraki 302-0024 Japan			

3-6 203	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS		
		<u>Terao</u>	<u>Yasuko</u>	
		<u>Ibaraki</u>	Japan <u>JPV</u>	Japan
	985-307, Oaza Onosaki, Tsukuba-shi, Ibaraki 305-0034 Japan			

4-6 204	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS		
		<u>Shintani</u>	<u>Yasushi</u>	
		<u>Ibaraki</u>	Japan <u>JPV</u>	Japan
	7-9-703, Kasuga 1-chome, Tsukuba-shi, Ibaraki 305-0821 Japan			

5-6 205	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS		
		<u>Hinuma</u>	<u>Shuji</u>	
		<u>Ibaraki</u>	Japan <u>JPV</u>	Japan
	7-9-1402, Kasuga 1-chome, Tsukuba-shi, Ibaraki 305-0821 Japan			

2006	FULL NAME OF INVENTOR	LAST NAME <u>Fukusumi</u>	FIRST NAME <u>Shoji</u>	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY <u>Ibaraki</u>	STATE OR FOREIGN COUNTRY Japan <u>JP</u>	COUNTRY OF CITIZENSHIP Japan
	POST OFFICE ADDRESS	POST OFFICE ADDRESS 17-6-302, Namiki 3-chome, Tsukuba-shi, Ibaraki 305-0044 Japan		

2007	FULL NAME OF INVENTOR	LAST NAME <u>Fujii</u>	FIRST NAME <u>Ryo</u>	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY <u>Ibaraki</u>	STATE OR FOREIGN COUNTRY Japan <u>JP</u>	COUNTRY OF CITIZENSHIP Japan
	POST OFFICE ADDRESS	POST OFFICE ADDRESS 7-9-303, Kasuga 1-chome, Tsukuba-shi, Ibaraki 305-0821 Japan		

2008	FULL NAME OF INVENTOR	LAST NAME <u>Hosoya</u>	FIRST NAME <u>Masaki</u>	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY <u>Ibaraki</u>	STATE OR FOREIGN COUNTRY Japan <u>JP</u>	COUNTRY OF CITIZENSHIP Japan
	POST OFFICE ADDRESS	POST OFFICE ADDRESS 711-83, Itaya 1-chome, Tsuchiura-shi, Ibaraki 300-0007 Japan		

2009	FULL NAME OF INVENTOR	LAST NAME <u>Kitada</u>	FIRST NAME <u>Chieko</u>	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY <u>Osaka</u>	STATE OR FOREIGN COUNTRY Japan <u>JA</u>	COUNTRY OF CITIZENSHIP Japan
	POST OFFICE ADDRESS	POST OFFICE ADDRESS 2-8, Minamikoyochi 1-cho, Sakai-shi, Osaka 590-0073 Japan		

210	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
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SEQUENCE LISTING

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<213> Artificial Sequence

<220>
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28

<210> 29
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<220>
 <223> Description of Artificial Sequence: Synthetic peptide

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<210> 60
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<220>
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 1 5

<210> 63
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